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<p>(21) International Application Number: PCT/US92/03825 (22) International Filing Date: 8 May 1992 (08.05.92) (30) Priority data: 698,709 10 May 1991 (10.05.91) US 773,229 9 October 1991 (09.10.91) US (71) Applicant: THE SALK INSTITUTE FOR BIOLOGICAL STUDIES [US/US]; 10010 North Torrey Pines Road, La Jolla, CA 92037 (US). (72) Inventors: MATHEWS, Lawrence, S. ; 3705 Coconino Court, San Diego, CA 92177 (US). VALE, Wylie, W. ; 1643 Valdez, La Jolla, CA 92037 (US).</p>	<p>(74) Agent: REITER, Stephen, R.; Pretty, Schroeder, Brueggemann & Clark, 444 South Flower Street, Suite 2000, Los Angeles, CA 90071 (US). (81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), MC (European patent), NL (European patent), SE (European patent). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>	
<p>(54) Title: CLONING AND RECOMBINANT PRODUCTION OF RECEPTOR(S) OF THE ACTIVIN/TGF-β SUPER-FAMILY</p>		
<p>(57) Abstract</p> <p>In accordance with the present invention, there are provided novel receptor proteins characterized by having the following domains, reading from the N-terminal end of said protein: an extracellular, ligand-binding domain, a hydrophobic, trans-membrane domain, and an intracellular, receptor domain having serine kinase-like activity. The invention receptors optionally further comprise a second hydrophobic domain at the amino terminus thereof. The invention receptor proteins are further characterized by having sufficient binding affinity for at least one member of the activin/TGF-β superfamily of polypeptide growth factors such that concentrations of ≤ 10 nM of said polypeptide growth factor occupy ≥ 50 % of the binding sites of said receptor protein. A presently preferred member of the invention superfamily of receptors binds specifically to activins, in preference to inhibins, transforming growth factor-β, and other non-activin-like proteins. DNA sequences encoding such receptors, assays employing same, as well as antibodies derived therefrom, are also disclosed.</p>		

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CLONING AND RECOMBINANT PRODUCTION
OF RECEPTOR(S) OF THE ACTIVIN/TGF- β SUPERFAMILY

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This invention was made with Government support
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National Institutes of Health. The Government has certain
rights in this invention.

FIELD OF THE INVENTION

10

The present invention relates to receptor
proteins, DNA sequences encoding same, and various uses
therefor.

15

BACKGROUND OF THE INVENTION

Activins are dimeric proteins which have the
ability to stimulate the production of follicle stimulating
hormone (FSH) by the pituitary gland. Activins share a
20 common subunit with inhibins, which inhibit FSH secretion.

Activins are members of a superfamily of
polypeptide growth factors which includes the inhibins, the
transforming growth factors- β (TGF- β), Mullerian duct
25 inhibiting substance, the Drosophila decapentaplegic
peptide, several bone morphogenetic proteins, and the
Vg-related peptides.

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As a result of their extensive anatomical distribution and multiple biological actions, members of this superfamily of polypeptide growth factors are believed to be involved in the regulation of numerous biological processes. Activin, for example, is involved in the proliferation of many tumor cell lines, the control of secretion and expression of the anterior pituitary hormones (e.g., FSH, GH and ACTH), neuron survival, hypothalamic oxytocin secretion, erythropoiesis, placental and gonadal steroidogenesis, early embryonic development, and the like.

Other members of the activin/TGF- β superfamily of polypeptide growth factors are involved in the regulation of cell function and cell proliferation for numerous cell types, in adults and embryos. For example, cells which are subject to regulation by one or more members of the activin/TGF- β superfamily of polypeptide growth factors include mesenchymal cells, muscle cells, skeletal cells, immune cells, hematopoietic cells, steroidogenic cells, endothelial cells, liver cells, epithelial cells, and the like.

Chemical cross-linking studies with a number of cell types suggests that multiple binding sites (i.e., receptors) exist on the surface of cells. However, little is known about the structure of these receptors, or about the second messenger signalling systems that they employ. It would be desirable, therefore, if the nature of these poorly characterized receptor proteins could be more fully understood.

BRIEF DESCRIPTION OF THE INVENTION

In accordance with the present invention, we have identified and characterized members of a new superfamily of receptor proteins which comprise three distinct domains: an extracellular, ligand-binding domain, a hydrophobic,

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trans-membrane domain, and an intracellular, receptor domain having serine kinase-like activity.

Also provided are DNAs encoding the above-described receptor proteins, and antibodies thereto, as well as bioassays, therapeutic compositions containing such proteins and/or antibodies, and applications thereof.

The DNAs of the invention are useful as probes for the identification of additional members of the invention superfamily of receptor proteins, and as coding sequences which can be used for the recombinant expression of the invention receptor proteins, or functional fragments thereof. The invention receptor proteins, and antibodies thereto, are useful for the diagnosis and therapeutic management of carcinogenesis, wound healing, disorders of the immune, reproductive, or central nervous systems, and the like.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a schematic diagram of receptors of the invention and the various domains thereof.

Figure 2 outlines the strategy used for expression cloning of a receptor of the activin/TGF- β receptor superfamily.

Figure 3 is a schematic of two mouse activin receptor clones. The top line of the figure is a restriction map, in kb, of mActR1 and mActR2, with numbering starting from bp 1 of mActR2. The dotted line in the figure represents 5' untranslated sequences present only in mActR1. The middle lines present a schematic representation of two activin receptor cDNA clones. Boxes represent coding sequences---black is the signal peptide, white is the extracellular ligand-binding domain, gray is

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the transmembrane, and the intracellular kinase domain is hatched. Amino acids are numbered beneath the schematics.

Figure 4 presents a comparison between activin
5 receptor and daf-1 [a *C. elegans* gene encoding a putative
receptor protein kinase (with unknown ligand); see Georgi,
et al., *Cell* 61: 635-645 (1990)]. Conserved residues
between the activin receptor and daf-1 are highlighted;
conserved kinase domain residues are designated with an
10 "**".

Figure 5A summarizes results of ¹²⁵I activin A
binding to COS cells transfected with pmActR1. Binding was
competed with unlabeled activin A. For the runs reported
15 herein, total binding was 4.6% of input cpm, non-specific
binding was 0.9% of input cpm, and therefore the specific
binding was 3.7% of input cpm. Data are shown as %
specific binding, normalized to 100%. The inset presents
a Scatchard analysis of the data [*Ann. NY Acad. Sci.* 51:
20 660-672 (1979)].

Figure 5B summarizes results of ¹²⁵I activin A
binding to COS cells transfected with pmActR2. Binding was
competed with unlabeled factors as indicated in the figure.
25 For the runs reported herein, total binding was 3.4% of
input cpm, non-specific binding was 0.9% of input cpm, and
therefore the specific binding was 2.5% of input cpm. Data
are shown as % specific binding, normalized to 100%.

Figure 6 is a phylogenetic tree, comparing the
30 relationship of the activin receptor kinase domain to other
protein kinases. To construct the tree, the catalytic
domains of representative sequences were empirically
aligned and evolutionary relatedness was calculated using
35 an algorithm designed by Fitch and Margoliash [*Science* 155:
279-284 (1967)], as implemented by Feng and Doolittle [*J.*
Mol. Evol. 25: 351-360 (1987)]. Known subfamilies of

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kinases are indicated in the figure. For those sequences that had similarity scores (i.e., a relative sequence identity) of at least 4 standard deviations above the mean (in comparison with all other known kinase sequences), the percent identity with the activin receptor is indicated. For further detail on kinase sequences, the reader is referred to Hanks and Quinn, Meth. Enzymol. 200: 38-62 (1991).

10 DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, there is provided a novel superfamily of receptor protein(s) characterized by having the following domains, reading from the N-terminal end of said protein:

15 an extracellular, ligand-binding domain,
 a hydrophobic, trans-membrane domain, and
 an intracellular domain having serine kinase-like activity.

20

The novel receptor protein(s) of the invention optionally further comprise a second hydrophobic domain at the amino terminus thereof.

25 As employed herein, the phrase "extracellular, ligand-binding domain" refers to that portion of receptors of the invention which has a high affinity for ligand, and which, when associated with a cell, resides primarily outside of the cell membrane. Because of its location, this domain is not exposed to the processing machinery present within the cell, but is exposed to all components of the extracellular medium. See Figure 1.

35 As employed herein, the phrase "hydrophobic, trans-membrane domain" refers to that portion of receptors of the invention which traverses the cell membrane, and serves as a "bridge" between the extracellular and

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intracellular domains of the receptor. The hydrophobic nature of this domain serves to anchor the receptor to the cell membrane. See Figure 1.

5 As employed herein, the phrase "intracellular domain having serine kinase-like activity" refers to that portion of receptors of the invention which resides within the cytoplasm, and which embodies the catalytic functionality characteristic of all receptors of the
10 invention. See Fig 1.

The optional second hydrophobic domain, positioned at the amino terminus of receptors of the invention, comprises a secretion signal sequence which
15 promotes the intracellular transport of the initially expressed receptor protein across the Golgi membrane. See Figure 1.

Members of the invention superfamily of receptors
20 can be further characterized as having sufficient binding affinity for at least one member of the activin/TGF- β superfamily of polypeptide growth factors such that concentrations of ≤ 10 nM of said polypeptide growth factor occupy $\geq 50\%$ of the binding sites of said receptor protein.
25

Binding affinity (which can be expressed in terms of association constants, K_a , or dissociation constants, K_d) refers to the strength of interaction between ligand and receptor, and can be expressed in terms of the
30 concentration of ligand necessary to occupy one-half (50%) of the binding sites of the receptor. A receptor having a high binding affinity for a given ligand will require the presence of very little ligand to become at least 50% bound (hence the K_d value will be a small number); conversely,
35 receptor having a low binding affinity for a given ligand will require the presence of high levels of ligand to become 50% bound (hence the K_d value will be a large

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number).

Reference to receptor protein "having sufficient binding affinity such that concentrations of said polypeptide growth factor less than or equal to 10 nM (i.e., ≤ 10 nM) occupy $\geq 50\%$ (i.e., greater than or equal to one-half) of the binding sites of said receptor protein" means that ligand (i.e., polypeptide growth factor) concentration(s) of no greater than about 10 nM are required in order for the ligand to occupy at least 50% of the active sites of said receptor, with much lower ligand concentrations typically being required. Presently preferred receptors of the present invention have a binding affinity such that ligand concentration(s) in the range of only about 100 - 500 pM are required in order to occupy (or bind to) at least 50% of the receptor binding sites.

Members of the invention superfamily of receptors can be divided into various subclasses, based on the approximate size of the crosslinked complexes obtained when radiolabeled activin is chemically crosslinked to cell extracts [see, for example, Example VI below, or Mathews and Vale in Cell 65:973-982 (1991)]. Type I activin/TGF- β receptors are those which form a crosslinked complex of about 65 kD with activin; Type II receptors are those which form a crosslinked complex of about 80-85 kD with activin; while Type III, Type IV and the like receptors are those which form crosslinked complexes with activin having molecular weights greater than about 100 kD.

Each member of a given subclass is related to other members of the same subclass by the high degree of homology (e.g., $>80\%$ overall amino acid homology; frequently having $>90\%$ overall amino acid homology) between such receptors; whereas members of a given subclass differ from members of a different subclass by the lower degree of homology (e.g., at least about 30% up to 80% overall amino

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acid homology; with in the range of about 40% up to 90% amino acid homology specifically in the kinase domains thereof) between such receptors. Typically, related receptors have at least 50% overall amino acid homology; with at least about 60% amino acid homology in the kinase domains thereof. Preferably, related receptors are defined as those which have at least 60% overall amino acid homology; with at least about 70% amino acid homology in the kinase domains thereof.

10

Based on the above criteria, the receptors described herein are designated Type II receptors, with the first discovered Type II receptor (i.e., the mouse-derived activin receptor) being designated ActRII, while subsequently identified Type II receptors which are not homologs of ActRII (because while clearly related by size and some sequence homology, they differ sufficiently to be considered as variants of ActRII), are designated ActRIIB, ActRIIC, etc.

20

Presently preferred members of the invention superfamily of receptors are further characterized by having a greater binding affinity for activins than for inhibins. Such receptors are frequently also observed to have:

25

substantially no binding affinity for transforming growth factors- β , and

substantially no binding affinity for non-activin-like proteins or compounds.

30

Additional members of the invention superfamily of receptors are further characterized by having a greater binding affinity for inhibins than for activins or TGF- β s.

35

Additional members of the invention superfamily of receptors are further characterized by having a greater binding affinity for TGF- β s than for activins or inhibins.

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As employed herein, "activin" refers to activin A (a homodimer of two inhibin β_A subunits), activin B (a homodimer of two inhibin β_B subunits), activin AB (a heterodimer composed of one inhibin β_A subunit and one
5 inhibin β_B subunit); "inhibin" refers to inhibin A (composed of the inhibin α subunit and an inhibin β_A subunit), inhibin B (composed of the inhibin α subunit and an inhibin β_B subunit); "transforming growth factor β or TGF- β " refers to TGF- β 1 (a homodimer of two TGF- β 1 subunits), TGF- β 2 (a
10 homodimer of two TGF- β 2 subunits), TGF- β 3 (a homodimer of two TGF- β 3 subunits), TGF- β 4 (a homodimer of two TGF- β 4 subunits), TGF- β 5 (a homodimer of two TGF- β 5 subunits), TGF- β 1.2 (a heterodimer of one TGF- β 1 subunit and one TGF- β 2 subunit), and the like.

15

Transforming growth factors- β (TGF- β s) are members of the activin/TGF- β superfamily of polypeptide growth factors. TGF- β s are structurally related to
20 activins, sharing at least 20-30% amino acid sequence homology therewith. TGF- β s and activins have a substantially similar distribution pattern of cysteine residues (or substitution) throughout the peptide chain. Furthermore, both polypeptides, in their active forms, are dimeric species.

25

As employed herein, the term "non-activin-like" proteins refers to any protein having essentially no structural similarity with activins (as defined broadly
30 herein).

30

Preferred members of the invention superfamily of receptors comprise those having in the range of about 500 amino acids, and are further characterized by having the following designated sizes for each of the domains thereof,
35 reading from the N-terminal end of said receptor:

the extracellular, ligand-binding domain preferably will have in the range of about 114-118

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amino acids,

the hydrophobic, trans-membrane domain preferably will have in the range of about 23-28 amino acids, beginning at the carboxy terminus of the extracellular domain, and

the intracellular domain having kinase-like activity preferably will have in the range of about 345-360 amino acids, beginning at the carboxy terminus of the hydrophobic, trans-membrane domain.

10

Receptors of the invention optionally further comprise a second hydrophobic domain having in the range of about 16-30 amino acids at the extreme amino terminus thereof (i.e., at the amino terminus of the extracellular, ligand-binding domain). This domain is a secretion signal sequence, which aids the transport of invention receptor(s) across the cell membrane. Exemplary secretion signal sequences include amino acids 1-19 of Sequence ID No. 1, amino acids 1-20 of Sequence ID No. 3, and the like. Such secretion signal sequences can be encoded by such nucleic acid sequences as nucleotides 71-127 of Sequence ID No. 1, nucleotides 468-527 of Sequence ID No. 3, and the like.

Members of the invention superfamily of receptors can be obtained from a variety of sources, such as, for example, pituitary cells, placental cells, hematopoietic cells, brain cells, gonadal cells, liver cells, bone cells, muscle cells, endothelial cells, epithelial cells, mesenchymal cells, kidney cells, and the like. Such cells can be derived from a variety of organisms, such as, for example, human, mouse, rat, ovine, bovine, porcine, frog, chicken, fish, mink, and the like.

Presently preferred amino acid sequences encoding receptor proteins of the invention include the sequence set forth in Sequence ID No. 2 (which represents a mouse activin receptor amino acid sequence), a modified form of

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Sequence ID No. 2 wherein the arginine at residue number 39 is replaced by a lysine, the isoleucine at residue number 92 is replaced by a valine, and the glutamic acid at residue number 288 is replaced by a glutamine (which modified form of Sequence ID No. 1 is referred to hereinafter as "Sequence ID No. 1'", and represents a human activin receptor amino acid sequence), and the sequence set forth as Sequence ID No. 4 (which represents a *Xenopus* activin receptor amino acid sequence), as well as functional, modified forms thereof. Those of skill in the art recognize that numerous residues of the above-described sequences can be substituted with other, chemically, sterically and/or electronically similar residues without substantially altering the biological activity of the resulting receptor species.

In accordance with another embodiment of the present invention, there is provided a soluble, extracellular, ligand-binding protein, further characterized by:

having sufficient binding affinity for at least one member of the activin/TGF- β superfamily of polypeptide growth factors such that concentrations of ≤ 10 nM of said polypeptide growth factor occupy $\geq 50\%$ of the binding sites on said receptor protein, and

having at least about 30% sequence identity with respect to:

the sequence of amino acids 20-134 set forth in Sequence ID No. 2;

the sequence of amino acids 20-134 set forth in Sequence ID No. 2, wherein the arginine residue at position number 39 is replaced by a lysine, and the isoleucine at residue number 92 is replaced by a valine; or

the sequence of amino acids 21-132 set forth in Sequence ID No. 4.

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Presently preferred soluble, extracellular, ligand-binding proteins contemplated by the present invention can be further characterized by having at least about 50% sequence identity with respect to:

5 the sequence of amino acids 20-134 set forth in Sequence ID No. 2;

 the sequence of amino acids 20-134 set forth in Sequence ID No. 2, wherein the arginine residue at position number 39 is replaced by a lysine, and the
10 isoleucine at residue number 92 is replaced by a valine; or

 the sequence of amino acids 21-132 set forth in Sequence ID No. 4;

15 with the presently most preferred soluble, extracellular, ligand-binding proteins having at least about 80% sequence identity with respect to the above-referenced fragments of Sequence ID Nos. 2 or 4 .

 Members of the class of soluble, ligand-binding
20 proteins contemplated by the present invention may be divided into various subclasses, as previously described, wherein members of one subclass may have a greater binding affinity for activins than for inhibins and/or TGF- β s; or alternatively, members of another subclass may have a
25 greater binding affinity for inhibins than for activins and/or TGF- β s; or alternatively, members of yet another subclass may have a greater binding affinity for TGF- β s than for activins and/or inhibins. It is, of course, understood by those of skill in the art, that members of
30 more than one subclass may have a greater binding affinity for one member of the activin/TGF- β superfamily of polypeptide growth factors, relative to other members of the superfamily.

35 Presently preferred soluble, extracellular, ligand-binding proteins of the present invention are further characterized by:

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having a greater binding affinity for activins than for inhibins,

having substantially no binding affinity for transforming growth factors- β , and

5 having substantially no binding affinity for non-activin-like proteins.

Presently preferred soluble, extracellular, ligand-binding proteins of the present invention typically
10 comprise in the range of about 114-118 amino acids.

Especially preferred soluble, extracellular, ligand-binding proteins of the invention are those having substantially the same amino acid sequence as that set
15 forth as:

residues 20-134 of Sequence ID No. 2;

residues 20-134 of Sequence ID No. 2, wherein the arginine residue at position number 39 is replaced by a lysine, and the isoleucine at residue number 92 is
20 replaced by a valine; or

residues 21-132 of Sequence ID No. 4.

As employed herein, the term "substantially the same amino acid sequence" refers to amino acid sequences
25 having at least about 80% identity with respect to the reference amino acid sequence, and will retain comparable functional and biological properties characteristic of the protein encoded by the reference amino acid. Preferably, proteins having "substantially the same amino acid
30 sequence" will have at least about 90% amino acid identity with respect to the reference amino acid sequence; with greater than about 95% amino acid sequence identity being especially preferred.

35 The above-described soluble proteins can be employed for a variety of therapeutic uses, e.g., to block receptors of the invention from affecting processes which

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the receptors would otherwise mediate. The presence of the soluble proteins of the invention will compete with functional ligand for the receptor, preventing the formation of a functional receptor-ligand complex, thereby
5 blocking the normal regulatory action of the complex.

In accordance with yet another embodiment of the present invention, there are provided antibodies generated against the above-described soluble proteins and receptor
10 proteins. Such antibodies can be employed for diagnostic applications, therapeutic applications, and the like. Preferably, for therapeutic applications, the antibodies employed will be monoclonal antibodies.

15 The above-described antibodies can be prepared employing standard techniques, as are well known to those of skill in the art, using the invention receptor proteins as antigens for antibody production.

20 In accordance with still another embodiment of the present invention, there are provided methods for modulating the transcription trans-activation of receptor(s) of the invention by contacting said receptor(s) with a modulating, effective amount of the above-described
25 antibodies.

The soluble proteins of the invention, and the antibodies of the invention, can be administered to a subject employing standard methods, such as, for example,
30 by intraperitoneal, intramuscular, intravenous, or subcutaneous injection, implant or transdermal modes of administration, and the like. In addition, methods such as transfection with viral or retroviral vectors encoding the invention compositions. One of skill in the art can
35 readily determine dose forms, treatment regiments, etc, depending on the mode of administration employed.

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In accordance with a further embodiment of the present invention, there are provided DNA sequences which encode the above-described soluble proteins and receptor proteins. Optionally, such DNA sequences, or fragments thereof, can be labeled with a readily detectable substituent (to be used, for example, as a hybridization probe).

The above-described receptor(s) can be encoded by numerous DNA sequences, e.g., a DNA sequence having a contiguous nucleotide sequence substantially the same as:

nucleotides 128 - 1609 of Sequence ID No. 1 (which encodes a mouse activin receptor);

variations of nucleotides 128 - 1609 of Sequence ID No. 1, wherein the codon for residue number 39 of the encoded amino acid codes for lysine, the codon for residue number 92 of the encoded amino acid codes for valine, and the codon for residue number 288 of the encoded amino acid encodes glutamine (which encodes a human activin receptor);

nucleotides 528 - 1997 of Sequence ID No. 3 (which encodes a Xenopus activin receptor); or

variations of any of the above sequences which encode the same amino acid sequences, but employ different codons for some of the amino acids.

As employed herein, the term "substantially the same as" refers to DNA having at least about 70% homology with respect to the nucleotide sequence of the DNA fragment with which subject DNA is being compared. Preferably, DNA "substantially the same as" a comparative DNA will be at least about 80% homologous to the comparative nucleotide sequence; with greater than about 90% homology being especially preferred.

Another DNA which encodes a receptor of the invention is one having a contiguous nucleotide sequence

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substantially the same as:

- nucleotides 71 - 1609 of Sequence ID No. 1 (which encodes a precursor-form of a mouse activin receptor);
- 5 variations of nucleotides 71 - 1609 of Sequence ID No. 1, wherein the codon for residue number 39 of the encoded amino acid codes for lysine, the codon for residue number 92 of the encoded amino acid codes for valine, and the codon for residue number 288 of the encoded amino acid encodes glutamine (which encodes a precursor-form of a human activin receptor);
- 10 nucleotides 468 - 1997 of Sequence ID No. 3 (which encodes a precursor form of a Xenopus activin receptor); or
- 15 variations of any of the above sequences which encode the same amino acid sequences, but employ different codons for some of the amino acids.

Yet another DNA which encodes the above-described receptor is one having a contiguous nucleotide sequence

20 substantially the same as set forth in Sequence ID No. 1, Sequence ID No. 1' or Sequence ID No. 3.

In accordance with a further embodiment of the present invention, the receptor-encoding cDNAs can be

25 employed to probe library(ies) (e.g., cDNA, genomic, and the like) for additional sequences encoding novel receptors of the activin/TGF- β superfamily. Such screening is initially carried out under low-stringency conditions, which comprise a temperature of less than about 42°C, a

30 formamide concentration of less than about 50%, and a moderate to low salt concentration. Presently preferred conditions for such screening comprise a temperature of about 37°C, a formamide concentration of about 20%, and a salt concentration of about 5X standard saline citrate

35 (SSC; 20X SSC contains 3M sodium chloride, 0.3M sodium citrate, pH 7.0). Such conditions will allow the identification of sequences which have a substantial degree

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of similarity with the probe sequence, without requiring perfect homology for the identification of a stable hybrid. The phrase "substantial similarity" refers to sequences which share at least 50% homology. Preferably, hybridization conditions will be selected which allow the identification of sequences having at least 70% homology with the probe, while discriminating against sequences which have a lower degree of homology with the probe.

10 In accordance with yet another embodiment of the present invention, there is provided a method for the recombinant production of receptor(s) of the invention by expressing the above-described DNA sequences in suitable host cells.

15 The use of a wide variety of recombinant organisms has been described for the production of peptides. One of skill in the art can readily determine suitable hosts (and expression conditions) for use in the recombinant production of the peptides of the present invention. Yeast hosts, bacterial hosts, mammalian hosts, and the like can be employed. Regulatory sequences capable of controlling the expression of invention peptides are well known for each of these host systems, as are growth conditions under which expression occurs.

20 In accordance with a further embodiment of the present invention, there is provided a binding assay employing receptors of the invention, whereby a large number of compounds can be rapidly screened to determine which compounds, if any, are capable of binding to the receptors of the invention. Then, more detailed assays can be carried out with those compounds found to bind, to further determine whether such compounds act as agonists or antagonists of invention receptors.

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Another application of the binding assay of the invention is the assay of test samples (e.g., biological fluids) for the presence or absence of members of the activin/TGF- β superfamily of polypeptide growth factors.

5 Thus, for example, serum from a patient displaying symptoms related to pathway(s) mediated by members of the activin/TGF- β superfamily of polypeptide growth factors can be assayed to determine if the observed symptoms are perhaps caused by over- or under-production of such

10 polypeptide growth factor.

The binding assays contemplated by the present invention can be carried out in a variety of ways, as can readily be identified by one of skill in the art. For

15 example, competitive binding assays can be employed, as well as radioimmunoassays, ELISA, ERMA, and the like.

In accordance with a still further embodiment of the present invention, there are provided bioassays for

20 evaluating whether test compounds are capable of acting as agonists or antagonists of receptor(s) of the present invention.

The bioassays of the present invention involve

25 evaluating whether test compounds are capable of acting as either agonists or antagonists for members of the invention superfamily of receptors, or functional modified forms of said receptor protein(s). The bioassay for evaluating whether test compounds are capable of acting as agonists

30 comprises:

(a) culturing cells containing:

DNA which expresses said receptor protein(s) or functional modified forms of said receptor protein(s), and

35 DNA encoding a hormone response element operatively linked to a reporter gene; wherein said culturing is carried out in the

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presence of at least one compound whose ability to induce transcription activation activity of receptor protein is sought to be determined, and thereafter

- 5 (b) monitoring said cells for expression of the product of said reporter gene.

The bioassay for evaluating whether test compounds are capable of acting as antagonists for
10 receptor(s) of the invention, or functional modified forms of said receptor(s), comprises:

- (a) culturing cells containing:

DNA which expresses said receptor protein(s), or functional modified forms of
15 said receptor protein(s), and

DNA encoding a hormone response element operatively linked to a reporter gene wherein said culturing is carried out in the presence of:

20 increasing concentrations of at least one compound whose ability to inhibit transcription activation of said receptor protein(s) is sought to be determined, and
a fixed concentration of at least one
25 agonist for said receptor protein(s), or functional modified forms of said receptor protein(s); and thereafter

- (b) monitoring in said cells the level of expression
30 of the product of said reporter gene as a function of the concentration of said compound, thereby indicating the ability of said compound to inhibit activation of transcription.

Host cells contemplated for use in the
35 bioassay(s) of the present invention, include CV-1 cells, COS cells, and the like; reporter and expression plasmids employed typically also contain the origin of replication

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of SV-40; and the reporter and expression plasmids employed also typically contain a selectable marker.

The hormone response element employed in the
5 bioassay(s) of the present invention can be selected from, for example, mouse mammary tumor virus long terminal repeat (MTV LTR), mammalian growth hormone promoter, and the reporter gene can be selected from chloramphenicol acetyltransferase (CAT), luciferase, β -galactosidase, and
10 the like.

The cells can be monitored for the level of expression of the reporter gene in a variety of ways, such as, for example, by photometric means [e.g., by colorimetry
15 (with a colored reporter product such as β -galactosidase), by fluorescence (with a reporter product such as luciferase), etc], by enzyme activity, and the like.

Compounds contemplated for screening in
20 accordance with the invention bioassays include activin- or TGF- β -like compounds, as well as compounds which bear no particular structural or biological relatedness to activin or TGF- β .

25 As employed herein, the phrase "activin- or TGF- β -like compounds" includes substances which have a substantial degree of homology (at least 20% homology) with the amino acid sequences of naturally occurring mammalian inhibin alpha and β_A or β_B chains (either singly or in any
30 combination) as well as alleles, fragments, homologs or derivatives thereof which have substantially the same qualitative biological activity as mammalian inhibin, activin, or TGF- β . Examples of activin- or TGF- β -like
35 compounds include activin A (a homodimer of two inhibin β_A subunits), activin B (a homodimer of two inhibin β_B subunits), activin AB (a heterodimer composed of one inhibin β_A subunit and one inhibin β_B subunit), inhibin A

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(composed of the inhibin α subunit and an inhibin β_A subunit), inhibin B (composed of the inhibin α subunit and an inhibin β_B subunit), TGF- β 1 (a homodimer of two TGF- β 1 subunits), TGF- β 2 (a homodimer of two TGF- β 2 subunits),
5 TGF- β 3 (a homodimer of two TGF- β 3 subunits), TGF- β 4 (a homodimer of two TGF- β 4 subunits), TGF- β 5 (a homodimer of two TGF- β 5 subunits), TGF- β 1.2 (a heterodimer of one TGF- β 1 subunit and one TGF- β 2 subunit), and the like.

10 Examples of compounds which bear no particular structural or biological relatedness to activin or TGF- β , but which are contemplated for screening in accordance with the bioassays of the present invention, include any
15 compound that is capable of either blocking the action of the invention receptor peptides, or promoting the action of the invention receptor peptides, such as, for example, alkaloids and other heterocyclic organic compounds, and the like.

20 The method employed for cloning the receptor(s) of the present invention involves expressing, in mammalian cells, a cDNA library of any cell type thought to respond to members of the activin/TGF- β superfamily of polypeptide growth factors (e.g., pituitary cells, placental cells,
25 fibroblast cells, and the like). Then, the ability of the resulting mammalian cells to bind a labeled receptor ligand (i.e., a labeled member of the activin/TGF- β superfamily of polypeptide growth factors) is determined. Finally, the desired cDNA insert(s) are recovered, based on the ability
30 of that cDNA, when expressed in mammalian cells, to induce (or enhance) the binding of labeled receptor ligand to said cell.

35 In addition to the above-described applications of the receptor proteins and DNA sequences of the present invention, the receptor or receptor-encoding compositions of the invention can be used in a variety of ways. For

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example, since activin is involved in many biological processes, the activin receptor (or antibodies thereto) can be applied to the modulation of such biological processes. For example, the stimulation of FSH release by activin can
5 either be enhanced (for example, by supplying the subject with increased amounts of the activin receptor, relative to the amount of endogenous receptor, e.g., by transfecting the subject with a tissue specific activin-encoding construct), or depressed (e.g., by administration to a
10 subject of antibodies to the activin receptor, thereby preventing formation of activin-receptor complex, which would then act to stimulate the release of FSH). Thus, the compositions of the present invention can be applied to the control of fertility in humans, domesticated animals, and
15 animals of commercial interest.

As another example, the effect of activin on mitosis of red and white blood cells can be modulated, for example, by administering to a subject (employing suitable
20 means of administration) a modulating, effective amount of activin receptor (which would enhance the ability of activin present in the cell to modulate mitosis). Alternatively, one could administer to a subject an
25 antibody to the activin receptor (or a portion thereof), which would reduce the effect of activin by blocking the normal interaction between activin and activin receptor.

As additional examples of the wide utility of the invention compositions, receptors and/or antibodies of the
30 invention can be used in such areas as the diagnosis and/or treatment of activin-dependent tumors, enhancing the survival of brain neurons, inducing abortion in livestock and other domesticated animals, inducing twinning in livestock and other domesticated animals, and so on.

35

As still further examples of the wide utility of the invention compositions, agonists identified for TGF- β

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specific receptors can be used to stimulate wound healing, to suppress the growth of TGF- β -sensitive tumors, to suppress immune response (and thereby prevent rejection of transplanted organs), and the like. Antagonists or the soluble, ligand-binding domain derived from TGF- β receptors can be used to block endogenous TGF- β , thereby promoting liver regeneration and stimulating some immune responses.

It can be readily seen, therefore, that the invention compositions have utility in a wide variety of diagnostic, clinical, veterinary and research applications.

The invention will now be described in greater detail by reference to the following non-limiting examples.

15

EXAMPLES

Recombinant human (rh) activin A, rh activin B, and rh inhibin A were generously provided by Genentech, Inc. Porcine TGF- β 1 was obtained from R+D Systems.

Double-stranded DNA was sequenced by the dideoxy chain termination method using the Sequenase reagents from US Biochemicals. Comparison of DNA sequences to databases was performed using the FASTA program [Pearson and Lipman, Proc. Natl. Acad. Sci. USA 85: 2444-2448 (1988)].

EXAMPLE I

Construction and Subdivision of cDNA Library

30

Polyadenylated RNA was prepared from AtT20 cells using the Fast Track reagents from InVitrogen. cDNA was commercially synthesized and ligated into the plasmid vector pCDNA1 using non-palindromic BstXI linkers, yielding a library of approximately 5×10^6 primary recombinants. The unamplified cDNA library was plated at 1000 clones per 100 mm plate, then scraped off the plates, frozen in glycerol

35

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and stored at -70° .

Activin suppresses adrenocorticotrophic hormone (ACTH) secretion by both primary anterior pituitary cell
5 cultures [Vale et al., Nature 321: 776-779 (1986)] and AtT20 mouse corticotropic cells. Because AtT20 cells possess activin receptors indistinguishable from those on other cell types (based on binding affinity measurements with activin A), these cells were chosen to be the source
10 of cDNA for transfection. A cDNA library of approximately 5×10^6 independent clones from AtT20 cells was constructed in the mammalian expression vector, pCDNA1, and screened using an expression cloning approach [Gearing et al., EMBO J. 8, 3667-3676 (1989)] based on the ability to detect activin
15 binding to single transfected cells. The library was divided into pools of 1000 clones, DNA was prepared from each pool of clones and transiently transfected into COS cells, and the cells screened for the capacity to bind iodinated activin A. Binding was assessed by performing
20 the transfections and binding reactions directly on chambered microscope slides, then dipping the slides in photographic emulsion and analyzing them under a microscope. Cells which had been transfected with an activin receptor cDNA, and consequently bound radioactive
25 activin, were covered with silver grains. DNA from pools of clones were analyzed either singly or in groups of three. Of 300 pools (approximately 300,000 clones) assayed in this manner, one group of three generated two positive cells when transfected into COS cells. The positive pool
30 (#64) was identified by transfecting and analyzing DNA from each pool of 1000 singly, and then was further fractionated until a single clone (pmActR1) was purified which generated $>10^4$ positive cells after transfection (see Table 1).

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Table 1
Purification of the activin receptor clone from
the AtT20 library

	<u>Pool</u>	<u>Clones/pool</u>	<u>Positive cells/slide</u>
5	62,63,64	3x1000	2
	64	1000	1-3
	64-51	400	4-10
	64-51-R10;64-51-C13	20	25-40
10	pmActR1	1	>10 ⁴

The total number of transfected cells capable of binding ¹²⁵I
activin A in a field of 2x10⁵ COS cells was counted for
15 pools of clones at each stage of the purification process.

pmActR1 contained a 1.7 kb insert, coding for a
protein of 342 amino acids (Figure 3); however, it was
incomplete on the 3' end, thus the last 17 amino acids were
20 encoded by vector sequences. In order to obtain the entire
sequence, the AtT20 library was rescreened by hybridization
with the 1.6 kb SacI-PstI fragment (Figure 3). Screening
6x10⁵ colonies yielded one additional positive clone
(pmActR2) which had a 2.6 kb insert and contained the
25 entire coding sequence for the mouse activin receptor
(Figure 3). The nucleic acid sequence and the deduced
amino acid sequence of the insert in pmActR2 are set forth
in Sequence ID No. 1.

30

EXAMPLE II
COS Cell Transfection

Aliquots of the frozen pools of clones were grown
overnight in 3 ml cultures of terrific broth, and mini-prep
35 DNA prepared from 1.5 ml using the alkaline lysis method
[Maniatis et al. Molecular Cloning (Cold Spring Harbor
Laboratory (1982))]. 1/10 of the DNA from a mini-prep (10

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1 ml of 100 ml) was used for each transfection.

2x10⁵ COS cells were plated on chambered microscope slides (1 chamber - Nunc) that had been coated with 20 µg/ml poly-D-lysine and allowed to attach for at least 3 hours. Cells were subjected to DEAE-Dextran mediated transfection as follows. 1.5 ml of serum-free Dulbecco's Modified Eagle's medium (DME) containing 100 mM chloroquine was added to the cells. DNA was precipitated in 200 ml DME/chloroquine containing 500 mg/ml DEAE-Dextran, then added to the cells. The cells were incubated at 37° for 4 hours, then the media was removed and the cells were treated with 10% DMSO in HEPES buffered saline for 2 minutes. Fresh media was added and the cells assayed 3 days later. For transfections with the purified clone, 2.5x10⁶ cells were transfected in 100 mm dishes with 5 µg purified DNA. The total transfection volume was 10 ml, and the DNA was precipitated in 400 µl.

20

EXAMPLE III
Binding Assay

Cells were washed 2x with HEPES buffered saline (HDB) containing 0.1% BSA, then incubated for 90 minutes at 22° in 0.5 ml HDB, 0.1% BSA containing 7x10⁵ cpm ¹²⁵I activin A (approximately 7 ng, 500 pM). The cells were then washed 3X with cold HDB, fixed for 15 minutes at 22° in 2.5% glutaraldehyde/HDB and washed 2X with HDB. The chambers were then peeled off the slides, and the slides dehydrated in 95% ethanol, dried under vacuum, dipped in NTB2 photographic emulsion (Kodak) and exposed in the dark at 4° for 3 days. Following development of the emulsion, the slides were dehydrated in 95% ethanol, stained with eosin and coverslipped with DPX mountant (Electron Microscopy Sciences). The slides were analyzed under darkfield illumination using a Leitz microscope.

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EXAMPLE IVSubdivision of Positive Pool

Of 300 pools screened (each pool containing about
5 1000 cDNAs), one positive pool (#64), which produced two
positive cells, was identified. Bacteria from the frozen
stock of this positive pool (#64) were replated at
approximately 400 clones per plate, replica plates were
made, and DNA was prepared from each subpool and analyzed
10 employing the binding assay described above. Several
positive subpools were found, which generated from 4-10
positive cells per slide. The bacteria from the replica
plate of one positive subpool were picked onto a grid, and
DNA prepared from pools of clones representing all the rows
15 and all the columns, as described by Wong [Science 228:810-
815 (1985)]. The identification of one positive row and
one positive column unambiguously identified a single
clone, which when transfected yielded $>10^4$ positive
cells/ 2×10^5 cells.

20

EXAMPLE VRadioreceptor Assay

10^5 COS cells transfected with either pmActR1 or
25 pmActR2, or 10^6 untransfected COS cells, were plated in 6
well dishes and allowed to grow overnight. The cells were
washed 2X with HDB, 0.1% BSA, and incubated at 22° for 90
minutes in 0.5 ml HDB, 0.1% BSA containing 100,000 cpm
(approximately 1 ng, 75 pM) 125 I activin A (5 μ g activin A
30 was iodinated by chloramine T oxidation to a specific
activity of 50-90 μ Ci/ μ g; iodinated activin A was purified
on a 0.7x20 cm G-25 column) and varying amounts of
unlabeled competitor hormone. Following binding, the cells
were washed 3X with cold HDB, solubilized in 0.5 ml 0.5 N
35 NaOH, removed from the dish and radioactivity was measured
in a gamma counter. Data presented in Figure 5 are
expressed as % specific binding, where 100% specific

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binding is the difference between binding in the absence of competitor and binding in the presence of a 100 fold molar excess of unlabeled activin A. Binding parameters were determined using the program LIGAND [Munson P.J. and Rodbard, D., Anal. Biochem. 107:220-259 (1980)].

EXAMPLE VI

Chemical Cross-linking

10 2×10^6 COS cells, or 5×10^6 AtT20 cells, were washed
2x with HDB, scraped off the dish, incubated for 90 minutes
at 22° under constant rotation in 0.5 ml HDB containing
 7×10^5 cpm (approximately 500 pM) 125 I activin A with or
without 500 ng (37 nM) unlabeled activin A. Cells were
15 diluted with 1 ml HDB, pelleted by centrifugation and
resuspended in 0.5 ml HDB. Disuccinimidyl suberate (DSS;
freshly dissolved in DMSO) was added to 500 μ M, and the
cells incubated at 0° for 30 minutes. The cross-linking
was terminated by addition of 1 ml 50 mM Tris-HCl pH 7.5,
20 100 mM NaCl, then the cells were pelleted by
centrifugation, resuspended in 100 μ l 50 mM Tris-HCl pH
7.5, 1% Triton X-100 and incubated at 0° for 60 minutes.
The samples were centrifuged 5 minutes at 13,000xg, and the
Triton-soluble supernatants analyzed by SDS-PAGE using 8.5%
25 polyacrylamide gels. The gels were dried and subjected to
autoradiography for 4-14 days.

EXAMPLE VII

RNA Blot Analysis

30 Total RNA was purified from tissue culture cells
and tissues using LiCl precipitation. 20 μ g total RNA was
run on 1.2% agarose, 2.2M formaldehyde gels, blotted onto
nylon membranes (Hybond - NEN), and hybridized with a 0.6
35 kb KpnI fragment (see Figure 3) which had been labeled with
 32 P by random priming using reagents from US Biochemicals.
Hybridization was performed at 42° in 50% formamide, and

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the filters were washed at 65° in 0.2X SSC.

EXAMPLE VIII

Sequence Analysis

5

Full length mouse activin receptor clone encodes a protein of 513 amino acids, with a 5' untranslated region of 70 bp and a 3' untranslated region of 951 bp. pmActR2 does not contain a poly A tail, although it does have a potential poladenylylation site at bp 2251. The insert in clone pmActR1 had an additional 551 bp of 5' untranslated sequence, was identical in the overlapping range, and stopped at the 3' end at base 1132 of pmActR2. The first methionine codon (ATG), at bp 71, in pmActR2 is in a favorable context for translation initiation [Kozak, M., Nucl. Acids Res. 15:8125-8148 (1987)], and is preceded by an in-frame stop codon. pmActR1 contains 3 additional ATGs in the 5' untranslated region; however, none of these is in an appropriate context for initiation, and all are followed by in-frame stop codons. While this unusually long 5' leader sequence may have functional significance, it is clearly not necessary for proper expression, because pmActR2, which lacks most of that sequence, can be functionally expressed in COS cells (see below).

25

Hydropathy analysis using the method of Kyte and Doolittle [J. Mol. Biol. 157:105-132 (1982)] revealed two hydrophobic regions: a 10 amino acid stretch at the amino terminus assumed to be a single peptide, and a single putative 26 residue membrane-spanning region between amino acids 119-142 (see Figure 1 and Sequence ID No. 2). The signal peptide contains the conserved n-, h- and c- domains common to signal sequences; the site of cleavage of the signal peptide, before Ala¹, is predicted based on rules described by von Heijne [Biochim. Biophys. Act. 947:307-333 (1988)]. As is common for the cytoplasmic side of membrane-spanning domains, the predicted transmembrane

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region is closely followed by two basic amino acids. The mature mouse activin receptor is thus predicted to be a 494 amino acid type I membrane protein of Mr 54 kDa, with a 116 amino acid N-terminal extracellular ligand binding domain, and a 346 amino acid intracellular signalling domain.

Comparison of the activin receptor sequence to the sequence databases revealed structural similarity in the intracellular domain to a number of receptor and non-receptor kinases. Analysis of the sequences of all kinases has led to the identification of a 300 amino acid kinase domain characterized by 12 subdomains containing a number of highly conserved amino acids [Hanks, S.K. and Quinn, A.M., Meth. Enzymol. 200:38-62 (1991) and Hanks et al., Science 241:42-52 (1988)]; the activin receptor sequence has all of these conserved subdomains in the proper order (Figure 4). A conserved Gly in subdomain I is replaced by Ala¹⁸⁰ in the activin receptor, but this residue has also been observed in other kinases. Based upon structural relatedness, therefore, this receptor is expected to be a functional protein kinase.

The sequences in two of these subdomains (VIB and VIII) can be used to predict tyrosine vs. serine/threonine substrate specificity [Hanks et al., (1988) supra]. The sequence of the mouse activin receptor in both of these subdomains is characteristic of serine kinases.

Table 2
Kinase Domain Predictive Sequences

<u>Subdomain</u>	<u>VIB</u>	<u>SEQ ID NO.</u>	<u>VIII</u>	<u>SEQ ID NO.</u>
serine kinase consensus	DLKPEN	5	G(T/S)XX(Y/F)X	6
activin receptor	DIKSKN	7	GTRRYM	8
tyrosine kinase consensus	DLAARN	9	XP(I/V)(K/R)W(T/M)	10

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Therefore, the activin receptor is expected to have serine/threonine specificity. Furthermore, the activin receptor does not have a tyrosine residue in the standard autophosphorylation region between subdomains VII and VIII, indicating that it is not a standard tyrosine kinase. The receptor could potentially autophosphorylate at Ser³³³ or Thr³³⁷. One interesting additional possibility is that the activin receptor kinase may have specificity for serine, threonine and tyrosine residues. Several kinases with these properties have recently been described [see, for example, Howell et al., *Mol. Cell. Biol.* 11:568-572 (1991), Stern et al., *Mol. Cell. Biol.* 11:987-1001 (1991) and Featherston, C. and Russell, P., *Nature* 349:808-811 (1991)].

15

Phylogenetic analysis of the activin receptor compared to 161 other kinase sequences revealed that the activin receptor and the *C.elegans* protein, *daf-1* [Georgi et al., *Cell* 61:635-645 (1990)] may constitute a separate subfamily of kinases (see Figure 6). *daf-1* is a putative transmembrane receptor involved in the developmental arrest of a non-feeding larval state and shares 32% identity with the activin receptor (see Figure 6). Like the activin receptor, *daf-1* is predicted to be a transmembrane serine/threonine-specific kinase; furthermore, both *daf* and the activin receptor have short, conserved inserts in the kinase domain sequence between subdomains VIA-VIB and X-XI that are not present in any other kinase (underlined in Figure 4B). This additional similarity lends credence to their belonging to a unique subfamily of kinases. The activin receptor is quite distantly related (18% amino acid sequence identity) to the only other known transmembrane serine/threonine protein kinase, encoded by the *ZmPK* gene of maize [Walker, J.C. and Zhang, R., *Nature* 345:743-746 (1990)].

35

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The extracellular domain of the activin receptor did not show similarity to any other sequences in the databases. This ligand binding domain is relatively small in comparison to those found in other growth factor
5 receptors, but like those receptors this domain has a high cysteine content. The pattern of these Cys residues, however, is not like either an immunoglobulin fold or the cysteine rich repeats of the EGF receptor. There are also
10 two potential sites of N-linked glycosylation in the extracellular domain, as well as a number of potential phosphorylation sites for protein kinase C and casein kinase II in the intracellular domain.

EXAMPLE IX

15 Binding Properties of the Cloned Activin Receptor

To verify that the cloned receptor is activin specific, competition binding experiments were performed on COS cells transiently transfected with either pmActR1 or
20 pmActR2. Cells transfected with either construct bound activin A with a single high affinity component ($K_d = 180$ pM; Figure 5), indicating that a functional (structurally complete) intracellular kinase domain is not required for ligand binding. This binding affinity is
25 consistent with that measured on other activin-responsive cell types [see, for example, Campen, C.A. and Vale, W., Biochem. Biophys. Res. Comm. 157:844-849 (1988); Hino et al., J. Biol. Chem. 264:10309-10314 (1989); Sugino et al., J. Biol. Chem. 263: 15249-15252 (1988); and Kondo et al.,
30 Biochem. Biophys. Res. Comm, 161:1267-1272 (1989)]. Untransfected COS cells do not bind activin A. The transfected cultures as a whole expressed approximately 26,000 receptors per cell; however, because only 15% of the cells express the transfected gene (as measured by
35 quantitating transfected cells as a fraction of all cells following dipping in emulsion), each transfected cell expressed an average of 175,000 receptors per cell. The

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level of expression per cell varies considerably, though, based on the number of accumulated silver grains. This value is comparable to the expression of other transfected cell surface proteins in COS cells.

5

Binding of iodinated activin A to COS cells transiently transfected with pmActR2 could be competed by activin B with slightly reduced potency compared to activin A; by inhibin A with approximately 10-fold lower potency; 10 and could not be competed by TGF- β 1 (Figure 5B). This affinity and specificity of binding match those observed following binding of activin A to a number of other activin-responsive cell types. Although activin B appears to bind the transfected receptor with lower affinity than 15 activin A, the activin B preparation used in these experiments may have suffered a reduction in potency, based on a comparison of bioactivity with activin A, since the recombinant synthesis of the activin B employed herein had been carried out some time ago [recombinant synthesis of 20 activin B is described by Mason et al., in Mol. Endocrinol. 3: 1352-1358 (1989)]. It is likely that this cDNA encodes a receptor for multiple forms of activin.

The size of the cloned activin receptor was 25 analyzed by affinity cross-linking 125 I activin A to COS cells transfected with pmActR2 using the bifunctional chemical cross-linker, disuccinimidyl suberate (DSS). A major cross-linked band of 84 kDa was observed in transfected, but not in untransfected cells. Subtracting 30 the molecular weight of activin, this represents a protein of 56 kDa, which corresponds well to the molecular weight predicted from the nucleic acid sequence data. Cross-linking 125 I activin A to AtT20 cells yields a major band of 65 kDa, with minor bands of approximately 78 and 84 kDa. 35 The size of the largest band matches that generated by the cloned receptor. The smaller bands could be either separate proteins, different phosphorylated forms of the

-35-

same protein, or degradation products of the full length clone; the sequences DKKRR at amino acid 35 and KKKR at amino acid 416 could be potential sites of proteolysis. Alternatively, these bands could come from alternatively
5 spliced products of the same gene.

The 84 and 65 kDa cross-linked bands have also been observed in other activin-responsive cell types [Hino, supra; Centrella et al., Mol. Cell. Biol. 11:250-258
10 (1991)], and interpreted to represent the signalling receptor, although complexes of other sizes have also been seen as well. The size of the activin receptor is very similar to a putative TGF- β receptor, to the limited extent it has been characterized by chemical cross-linking [see
15 Massague et al., Ann. N.Y. Acad. Sci. 593: 59-72 (1990)].

EXAMPLE X

Expression of Activin Receptor mRNA

20 The distribution of activin receptor mRNA was analyzed by Northern blot. Two mRNA species, of 6.0 and 3.0 kb, were observed in AtT20 cells as well as a number of mouse tissues, including brain, testis, pancreas, liver and kidney. The total combined size of the inserts from
25 pmActR1 and pmActR2 is 3.1 kb, which corresponds to the size of the smaller transcript. Neither the extent of similarity between the two mRNAs, nor the significance of having two transcripts is clear. The genes for several other hormone receptors have been shown to be alternatively
30 spliced to generate both a cell surface receptor and a soluble binding protein, and it is possible that the activin receptor is processed in a similar manner.

Interestingly, the relative abundance of the two
35 transcripts varies depending on the source. While AtT20 cells have approximately equal levels of both mRNAs, most tissues had much greater levels of the 6.0 kb transcript,

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with little or no expression of the 3.0 kb transcript. Testis, on the other hand, had a greater amount of the 3.0 kb band. Expression of activin receptor mRNA in brain, liver and testis is in accord with described biological actions of activin in those tissues [Mine et al., Endocrinol. 125:586-591 (1989); Vale et al., Peptide Growth Factors and Their Receptors, Handbook of Experimental Pharmacology, M.A. Sporn and A.B. Roberts, ed., Springer-Verlag (1990), in press].

10

EXAMPLE XI

Identification of a Human Activin Receptor

A human testis library (purchased from Clontech; catalog no. HL1010b) was probed with the full length mouse activin receptor gene (see Sequence ID No. 1) under the following conditions:

Hybridization stringency:

20% formamide, 6X SSC at 42°C;

Wash stringency:

2X SSC, 0.1% SDS at 42°C.

A sequence which is highly homologous with the mouse activin receptor was identified (Sequence ID No. 1'). Due to the high degree of homology between this receptor and the mouse activin receptor, this receptor is designated as the human form of the activin receptor from the same subclass as the mouse receptor described above.

30

EXAMPLE XII

Identification of a Xenopus Activin Receptor

A Xenopus stage 17 embryo cDNA library (prepared as described by Kintner and Melton in Development 99: 311-325 (1987) was probed with the full length mouse activin receptor gene (see Sequence ID No. 1) under the following conditions:

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Hybridization stringency:

20% formamide, 6X SSC at 42°C;

Wash stringency:

2X SSC, 0.1% SDS at 42°C.

5

A sequence having a substantial degree of homology with respect to the mouse activin receptor was identified (Sequence ID No. 3). The degree of overall amino acid homology (relative to the mouse acitvin
10 receptor) is only about 69% (with 77% homology in the intracellular domain and 58% homology in the extracellular domain). Due to the moderate degree of homology between this receptor and the mouse activin receptor, this receptor is designated as an activin receptor from a different
15 subclass than the mouse receptor described above.

EXAMPLE XIIIFunctional Assays of ActRs in Xenopus embryos

20 To determine whether xActRIIB can transmit a signal in response to activin, xActRIIB RNA was synthesized in vitro and injected into *Xenopus* embryos at two different concentrations. Injected embryos were allowed to develop to stage 9, at which time animal caps were dissected and
25 treated overnight with different concentrations of activin. The xActRIIB cDNA was cloned into rp64T [see Krieg and Melton in *Methods in Enzymology*, Abelson and Simon, Eds. (Academic Press, New York, 1987), vol. 155, p. 397] and transcribed in vitro to generate a capped, synthetic
30 xActRIIB RNA [see Melton et al., in *Nucleic Acids Res.* 12:7035 (1984) and Kintner in *Neuron* 1:545 (1988)]. Embryos at the two- to four-cell stage were injected with about 20 nl of RNA at concentrations of 0.02 ng/nl, or 0.1 ng/nl, spread between four quadrants of the animal pole.
35 At stage 9, animal caps were removed from RNA-injected embryos and incubated in 0.5x modified mammalian Ringer's (MMR), 0.1% bovine serum albumin (BSA) with different

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concentrations of purified, porcine activin A (six caps per incubation). After 20 hours in culture, total RNA was prepared.

5 The response of the caps to activin was assessed by quantifying muscle-specific actin RNA with a ribonuclease protection assay as per Blackwell and Weintraub, *Science* 250:1104 (1990). Embryos injected with 0.4 and 2.0 ng of xActRIIB RNA were approximately 10- and
10 100-fold more sensitive, respectively, to activin than control embryos. The low amount of muscle actin found in animal caps in the absence of added activin A is probably a consequence of contamination of the animal cap with a small amount of marginal zone tissue.

15 The amount of muscle actin decreased with increasing concentration of activin in the embryos injected with 2 ng of xActRIIB RNA. This is consistent with the observation that isolated animal cap cells uniformly
20 exposed to different concentrations of activin only form muscle cells in response to a narrow range of activin concentrations [see Blackmann and Kadesch in *Genes and Development* 5:1057 (1990)]. The present results indicate that the concentration of ligand and the amount of receptor
25 are both important in determining the signal transmitted. Thus, the range of activin concentrations that lead to muscle differentiation is lower in animal cap cells from injected embryos, which are expressing more receptor than normal, than from uninjected embryos.

30

EXAMPLE XIV

Analysis of kinase activity of mActRII

A fragment of cDNA corresponding to the entire
35 intracellular domain of mActRII (amino acids 143-494) was subcloned into the vector pGEX-2T [see Smith and Johnson in *Gene* 67:31-40 (1988)], creating a fusion protein between

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glutathione S-transferase (GST) and the putative kinase domain of the receptor. This plasmid was introduced into bacteria and the expressed fusion protein was purified using glutathione affinity chromatography as described by
5 Smith and Johnson. Approximately 100-200 ng of fusion protein, or of purified GST, were incubated with 25 μ Ci [γ -³²P] ATP in a buffer containing 50 mM Tris, 10 mM MgCl₂ for 30 minutes at 37°C. The products were analyzed by SDS-PAGE and autoradiography. The fusion protein, but not the GST
10 alone, became phosphorylated, indicating that the kinase domain of the fusion protein was functional. Phosphoamino acid analysis, performed according to Cooper et al. [Meth. Enzym. 99:387 (1983)], indicated that the predominant amino acid residue that became phosphorylated was threonine.

15

While the invention has been described in detail with reference to certain preferred embodiments thereof, it will be understood that modifications and variations are within the spirit and scope of that which is described and
20 claimed.

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SUMMARY OF SEQUENCES

Sequence ID No. 1 is the nucleic acid sequence (and the deduced amino acid sequence) of a cDNA encoding a mouse-derived activin receptor of the present invention.

Sequence ID No. 1' is a nucleic acid sequence encoding a human-derived activin receptor of the present invention. Sequence ID No. 1' is substantially the same as Sequence ID No. 1, except that the codon for amino acid residue number 39 encodes lysine (i.e., nucleotides 185-187 are AAA or AAG), the codon for amino acid residue 92 encodes valine (i.e., nucleotides 344-346 are GTN, wherein N is A, C, G or T), and the codon for amino acid residue number 288 encodes glutamine (i.e., nucleotides 932-934 are CAA or CAG).

Sequence ID No. 2 is the deduced amino acid sequence of a mouse-derived activin receptor of the present invention.

Sequence ID No. 2' is an amino acid sequence for a human-derived activin receptor of the present invention. Sequence ID No. 2' is substantially the same as Sequence ID No. 2, except that amino acid residue number 39 is lysine, amino acid residue 92 is valine, and amino acid residue number 288 is glutamine.

Sequence ID No. 3 is the nucleic acid sequence (and the deduced amino acid sequence) of a cDNA encoding a Xenopus-derived activin receptor of the present invention.

Sequence ID No. 4 is the deduced amino acid sequence of a Xenopus-derived activin receptor of the present invention.

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Sequence ID No. 5 is the amino acid sequence of the VIB subdomain of the serine kinase consensus sequence.

Sequence ID No. 6 is the amino acid sequence of the VIII subdomain of the serine kinase consensus sequence.

Sequence ID No. 7 is the amino acid sequence of the VIB subdomain of the invention activin receptor.

10 Sequence ID No. 8 is the amino acid sequence of the VIII subdomain of the invention activin receptor.

Sequence ID No. 9 is the amino acid sequence of the VIB subdomain of the tyrosine kinase consensus
15 sequence.

Sequence ID No. 10 is the amino acid sequence of the VIII subdomain of the tyrosine kinase consensus
20 sequence.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Mathews, Ph.D., Lawrence S.
Vale, Ph.D., Wylie W.
- (ii) TITLE OF INVENTION: CLONING AND RECOMBINANT PRODUCTION OF
RECEPTOR(S) OF THE ACTIVIN/TGF-BETA SUPERFAMILY
- (iii) NUMBER OF SEQUENCES: 10
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: PRETTY, SCHROEDER, BRUEGGEMANN & CLARK
 - (B) STREET: 444 South Flower Street, Suite 2000
 - (C) CITY: Los Angeles
 - (D) STATE: California
 - (E) COUNTRY: United States
 - (F) ZIP: 90071-2921
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: US
 - (B) FILING DATE: 08-MAY-1992
 - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Reiter, Mr., Stephen E.
 - (B) REGISTRATION NUMBER: 31192
 - (C) REFERENCE/DOCKET NUMBER: P31 9309/FP31 9291
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: (619) 546-4737
 - (B) TELEFAX: (619) 546-9392

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2563 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 71..1609

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```

CTCCGAGGAA GACCCAGGGA ACTGGATATC TAGCGAGAAC TTCCTACGGC TTCTCCGGCG      60
CCTCGGGAAA ATG GGA GCT GCT GCA AAG TTG GCG TTC GCC GTC TTT CTT      109
Met Gly Ala Ala Ala Lys Leu Ala Phe Ala Val Phe Leu
      1           5           10

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ATC TCT TGC TCT TCA GGT GCT ATA CTT GGC AGA TCA GAA ACT CAG GAG Ile Ser Cys Ser Ser Gly Ala Ile Leu Gly Arg Ser Glu Thr Gln Glu 15 20 25	157
TGT CTT TTC TTT AAT GCT AAT TGG GAA AGA GAC AGA ACC AAC CAG ACT Cys Leu Phe Phe Asn Ala Asn Trp Glu Arg Asp Arg Thr Asn Gln Thr 30 35 40 45	205
GGT GTT GAA CCT TGC TAT GGT GAT AAA GAT AAA CGG CGA CAT TGT TTT Gly Val Glu Pro Cys Tyr Gly Asp Lys Asp Lys Arg Arg His Cys Phe 50 55 60	253
GCT ACC TGG AAG AAT ATT TCT GGT TCC ATT GAA ATA GTG AAG CAA GGT Ala Thr Trp Lys Asn Ile Ser Gly Ser Ile Glu Ile Val Lys Gln Gly 65 70 75	301
TGT TGG CTG GAT GAT ATC AAC TGC TAT GAC AGG ACT GAT TGT ATA GAA Cys Trp Leu Asp Asp Ile Asn Cys Tyr Asp Arg Thr Asp Cys Ile Glu 80 85 90	349
AAA AAA GAC AGC CCT GAA GTG TAC TTT TGT TGC TGT GAG GGC AAT ATG Lys Lys Asp Ser Pro Glu Val Tyr Phe Cys Cys Cys Glu Gly Asn Met 95 100 105	397
TGT AAT GAA AAG TTC TCT TAT TTT CCG GAG ATG GAA GTC ACA CAG CCC Cys Asn Glu Lys Phe Ser Tyr Phe Pro Glu Met Glu Val Thr Gln Pro 110 115 120 125	445
ACT TCA AAT CCT GTT ACA CCG AAG CCA CCC TAT TAC AAC ATT CTG CTG Thr Ser Asn Pro Val Thr Pro Lys Pro Pro Tyr Tyr Asn Ile Leu Leu 130 135 140	493
TAT TCC TTG GTA CCA CTA ATG TTA ATT GCA GGA ATT GTC ATT TGT GEA Tyr Ser Leu Val Pro Leu Met Leu Ile Ala Gly Ile Val Ile Cys Ala 145 150 155	541
TTT TGG GTG TAC AGA CAT CAC AAG ATG GCC TAC CCT CCT GTA CTT GTT Phe Trp Val Tyr Arg His His Lys Met Ala Tyr Pro Pro Val Leu Val 160 165 170	589
CCT ACT CAA GAC CCA GGA CCA CCC CCA CCT TCC CCA TTA CTA GGG TTG Pro Thr Gln Asp Pro Gly Pro Pro Pro Ser Pro Leu Leu Gly Leu 175 180 185	637
AAG CCA TTG CAG CTG TTA GAA GTG AAA GCA AGG GGA AGA TTT GGT TGT Lys Pro Leu Gln Leu Leu Glu Val Lys Ala Arg Gly Arg Phe Gly Cys 190 195 200 205	685
GTC TGG AAA GCC CAG TTG CTC AAT GAA TAT GTG GCT GTC AAA ATA TTT Val Trp Lys Ala Gln Leu Leu Asn Glu Tyr Val Ala Val Lys Ile Phe 210 215 220	733
CCA ATA CAG GAC AAA CAG TCC TGG CAG AAT GAA TAT GAA GTC TAT AGT Pro Ile Gln Asp Lys Gln Ser Trp Gln Asn Glu Tyr Glu Val Tyr Ser 225 230 235	781
CTA CCT GGA ATG AAG CAT GAG AAC ATA CTA CAG TTC ATT GGT GCA GAG Leu Pro Gly Met Lys His Glu Asn Ile Leu Gln Phe Ile Gly Ala Glu 240 245 250	829
AAA AGA GGC ACC AGT GTG GAT GTG GAC CTG TGG CTA ATC ACA GCA TTT Lys Arg Gly Thr Ser Val Asp Val Asp Leu Trp Leu Ile Thr Ala Phe 255 260 265	877
CAT GAA AAG GGC TCA CTG TCA GAC TTT CTT AAG GCT AAT GTG GTC TCT His Glu Lys Gly Ser Leu Ser Asp Phe Leu Lys Ala Asn Val Val Ser 270 275 280 285	925

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TGG AAT GAA CTT TGT CAT ATT GCA GAA ACC ATG GCT AGA GGA TTG GCA Trp Asn Glu Leu Cys His Ile Ala Glu Thr Met Ala Arg Gly Leu Ala 290 295 300	973
TAT TTA CAT GAG GAT ATA CCT GGC TTA AAA GAT GGC CAC AAG CCT GCA Tyr Leu His Glu Asp Ile Pro Gly Leu Lys Asp Gly His Lys Pro Ala 305 310 315	1021
ATC TCT CAC AGG GAC ATC AAA AGT AAA AAT GTG CTG TTG AAA AAC AAT Ile Ser His Arg Asp Ile Lys Ser Lys Asn Val Leu Leu Lys Asn Asn 320 325 330	1069
CTG ACA GCT TGC ATT GCT GAC TTT GGG TTG GCC TTA AAG TTC GAG GCT Leu Thr Ala Cys Ile Ala Asp Phe Gly Leu Ala Leu Lys Phe Glu Ala 335 340 345	1117
GGC AAG TCT GCA GGT GAC ACC CAT GGG CAG GTT GGT ACC CGG AGG TAT Gly Lys Ser Ala Gly Asp Thr His Gly Gln Val Gly Thr Arg Arg Tyr 350 355 360 365	1165
ATG GCT CCA GAG GTG TTG GAG GGT GCT ATA AAC TTC CAA AGG GAC GCA Met Ala Pro Glu Val Leu Glu Gly Ala Ile Asn Phe Gln Arg Asp Ala 370 375 380	1213
TTT CTG AGG ATA GAT ATG TAC GCC ATG GGA TTA GTC CTA TGG GAA TTG Phe Leu Arg Ile Asp Met Tyr Ala Met Gly Leu Val Leu Trp Glu Leu 385 390 395	1261
GCT TCT CGT TGC ACT GCT GCA GAT GGA CCC GTA GAT GAG TAC ATG TTA Ala Ser Arg Cys Thr Ala Ala Asp Gly Pro Val Asp Glu Tyr Met Leu 400 405 410	1309
CCA TTT GAG GAA GAA ATT GGC CAG CAT CCA TCT CTT GAA GAT ATG CAG Pro Phe Glu Glu Glu Ile Gly Gln His Pro Ser Leu Glu Asp Met Gln 415 420 425	1357
GAA GTT GTT GTG CAT AAA AAA AAG AGG CCT GTT TTA AGA GAT TAT TGG Glu Val Val Val His Lys Lys Lys Arg Pro Val Leu Arg Asp Tyr Trp 430 435 440 445	1405
CAG AAA CAT GCA GGA ATG GCA ATG CTC TGT GAA ACG ATA GAA GAA TGT Gln Lys His Ala Gly Met Ala Met Leu Cys Glu Thr Ile Glu Glu Cys 450 455 460	1453
TGG GAT CAT GAT GCA GAA GCC AGG TTA TCA GCT GGA TGT GTA GGT GAA Trp Asp His Asp Ala Glu Ala Arg Leu Ser Ala Gly Cys Val Gly Glu 465 470 475	1501
AGA ATT ACT CAG ATG CAA AGA CTA ACA AAT ATC ATT ACT ACA GAG GAC Arg Ile Thr Gln Met Gln Arg Leu Thr Asn Ile Ile Thr Thr Glu Asp 480 485 490	1549
ATT GTA ACA GTG GTC ACA ATG GTG ACA AAT GTT GAC TTT CCT CCC AAA Ile Val Thr Val Val Thr Met Val Thr Asn Val Asp Phe Pro Pro Lys 495 500 505	1597
GAA TCT AGT CTA TGATGGTGGC ACCGTCTGTA CACACTGAGG ACTGGGACTC Glu Ser Ser Leu 510	1649
TGAACTGGAG CTGCTAAGCT AAGGAAAGTG CTTAGTTGAT TTTCTGTGTG AAATGAGTAG	1709
GATGCCTCCA GGACATGTAC GCAAGCAGCC CCTTGTGGAA AGCATGGATC TGGGAGATGG	1769
ATCTGGGAAA CTTACTGCAT CGTCTGCAGC ACAGATATGA AGAGGAGTCT AAGGAAAAG	1829
CTGCAAACTG TAAAGAACTT CTGAAAATGT ACTCGAAGAA TGTGGCCCTC TCCAAATCAA	1889

GGATCTTTTG GACCTGGCTA ATCAAGTATT TGCAAAACTG ACATCAGATT TCTTAATGTC 1949
 TGTCAGAAGA CACTAATTCC TTAAATGAAC TACTGCTATT TTTTTTAAAT GAAAAACTTT 2009
 TCATTTCAGA TTTTAAAAG GGTAACITTT TATTGCATTT GCTGTTGTTT CTATAAATGA 2069
 CTATTGTAAT GCCAACATGA CACAGCTTGT GAATGTGTAG TGTGCTGCTG TTCTGTGTAC 2129
 ATAGTCATCA AAGTGGGGTA CAGTAAAGAG GCTTCCAAGC ATTACTTTAA CCTCCCTCAA 2189
 CAAGGTATAC CTCAGTTCCA CGGTTGTTAA ATTATAAAAT TGAAAACACT AACAGAATTT 2249
 GAATAAATCA GTCCATGTTT TATAACAAGG TTAATTACAA ATTCACTGTG TTATTTAAGA 2309
 AAAAATGGTA AGCTATGCTT AGTGCCAATA GTAAGTGGCT ATTTGTAAG CAGTGTTTTA 2369
 GCTTTTCTTC TACTGGCTTG TAATTTAGGG AAAACAAGTG CTGTCTTTGA AATGGAAAAG 2429
 AATATGGTGT CACCCTACCC CCCATACTTA TATCAAGGTC CCAAAATATT CTTTTCCATT 2489
 TCAAAGACAG CACTTTGAAA ACCCTAAATT ACAAGCCAGT AGAAGAAAAG CTA AACACC 2549
 CTTTACAAAT AGCC 2563

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 513 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Gly Ala Ala Ala Lys Leu Ala Phe Ala Val Phe Leu Ile Ser Cys
 1 5 10 15
 Ser Ser Gly Ala Ile Leu Gly Arg Ser Glu Thr Gln Glu Cys Leu Phe
 20 25 30
 Phe Asn Ala Asn Trp Glu Arg Asp Arg Thr Asn Gln Thr Gly Val Glu
 35 40 45
 Pro Cys Tyr Gly Asp Lys Asp Lys Arg Arg His Cys Phe Ala Thr Trp
 50 55 60
 Lys Asn Ile Ser Gly Ser Ile Glu Ile Val Lys Gln Gly Cys Trp Leu
 65 70 75 80
 Asp Asp Ile Asn Cys Tyr Asp Arg Thr Asp Cys Ile Glu Lys Lys Asp
 85 90 95
 Ser Pro Glu Val Tyr Phe Cys Cys Cys Glu Gly Asn Met Cys Asn Glu
 100 105 110
 Lys Phe Ser Tyr Phe Pro Glu Met Glu Val Thr Gln Pro Thr Ser Asn
 115 120 125
 Pro Val Thr Pro Lys Pro Pro Tyr Tyr Asn Ile Leu Leu Tyr Ser Leu
 130 135 140
 Val Pro Leu Met Leu Ile Ala Gly Ile Val Ile Cys Ala Phe Trp Val
 145 150 155 160

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Tyr Arg His His Lys Met Ala Tyr Pro Pro Val Leu Val Pro Thr Gln
 165 170 175

Asp Pro Gly Pro Pro Pro Pro Ser Pro Leu Leu Gly Leu Lys Pro Leu
 180 185 190

Gln Leu Leu Glu Val Lys Ala Arg Gly Arg Phe Gly Cys Val Trp Lys
 195 200 205

Ala Gln Leu Leu Asn Glu Tyr Val Ala Val Lys Ile Phe Pro Ile Gln
 210 215 220

Asp Lys Gln Ser Trp Gln Asn Glu Tyr Glu Val Tyr Ser Leu Pro Gly
 225 230 235 240

Met Lys His Glu Asn Ile Leu Gln Phe Ile Gly Ala Glu Lys Arg Gly
 245 250 255

Thr Ser Val Asp Val Asp Leu Trp Leu Ile Thr Ala Phe His Glu Lys
 260 265 270

Gly Ser Leu Ser Asp Phe Leu Lys Ala Asn Val Val Ser Trp Asn Glu
 275 280 285

Leu Cys His Ile Ala Glu Thr Met Ala Arg Gly Leu Ala Tyr Leu His
 290 295 300

Glu Asp Ile Pro Gly Leu Lys Asp Gly His Lys Pro Ala Ile Ser His
 305 310 315 320

Arg Asp Ile Lys Ser Lys Asn Val Leu Leu Lys Asn Asn Leu Thr Ala
 325 330 335

Cys Ile Ala Asp Phe Gly Leu Ala Leu Lys Phe Glu Ala Gly Lys Ser
 340 345 350

Ala Gly Asp Thr His Gly Gln Val Gly Thr Arg Arg Tyr Met Ala Pro
 355 360 365

Glu Val Leu Glu Gly Ala Ile Asn Phe Gln Arg Asp Ala Phe Leu Arg
 370 375 380

Ile Asp Met Tyr Ala Met Gly Leu Val Leu Trp Glu Leu Ala Ser Arg
 385 390 395 400

Cys Thr Ala Ala Asp Gly Pro Val Asp Glu Tyr Met Leu Pro Phe Glu
 405 410 415

Glu Glu Ile Gly Gln His Pro Ser Leu Glu Asp Met Gln Glu Val Val
 420 425 430

Val His Lys Lys Lys Arg Pro Val Leu Arg Asp Tyr Trp Gln Lys His
 435 440 445

Ala Gly Met Ala Met Leu Cys Glu Thr Ile Glu Glu Cys Trp Asp His
 450 455 460

Asp Ala Glu Ala Arg Leu Ser Ala Gly Cys Val Gly Glu Arg Ile Thr
 465 470 475 480

Gln Met Gln Arg Leu Thr Asn Ile Ile Thr Thr Glu Asp Ile Val Thr
 485 490 495

Val Val Thr Met Val Thr Asn Val Asp Phe Pro Pro Lys Glu Ser Ser
 500 505 510

Leu

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2335 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vii) IMMEDIATE SOURCE:

- (B) CLONE: XACTR

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 468..1997

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CCGCCCACAC AGTGCAGTGA ATAATAGCCG GTGCGGCCCC TCCCCTCTTT CCCTGGCAGT	60
TGTGTATCTG TCACATTGAA GTTTGGGCTC CTGTGAGTCT GAGCCTCCCC CTGTGTCTCA	120
TGTGAAGCTG CTGCTGCAGA AGGTGGAGTC GTTGCATGAG GGTGGGGGGA GTCGCTGCTG	180
TTTGATCTGC CTCTGCTCCC CATTACACT CTCATTTTCAT TCCCACGGAT CCACATTACA	240
ACTCGCCTTT AACCCCTTCC CTGGCGGAGC CCACGCGTCT TTCATCCCTC CTGCCGCGGC	300
CGCTGAGCGA CCAGAGCGCG ACATTGTGTC GCGGGGGGAT TGGGCGACAT TGTGCGAAT	360
AATCGGAGCT GCTGGGGGGG AACTGATACA ACGTTGCGAC TGTAAGGAA TTAACTCGGC	420
CGAATGGGAT TTTATCTGTG TCGGTGAGAG AAGCGGATCC CAGGAGC ATG GGG GCG	476
	Met Gly Ala
	1
TCT GTA GCG CTG ACT TTT CTA CTT CTT CTT GCA ACT TTC CGC GCA GGC	524
Ser Val Ala Leu Thr Phe Leu Leu Leu Leu Ala Thr Phe Arg Ala Gly	
5 10 15	
TCA GGA CAC GAT GAA GTG GAG ACA AGA GAG TGC ATC TAT TAC AAT GCC	572
Ser Gly His Asp Glu Val Glu Thr Arg Glu Cys Ile Tyr Tyr Asn Ala	
20 25 30 35	
AAC TGG GAA CTG GAG AAG ACC AAC CAA AGT GGG GTG GAA AGC TGC GAA	620
Asn Trp Glu Leu Glu Lys Thr Asn Gln Ser Gly Val Glu Ser Cys Glu	
40 45 50	
GGG GAA AAG GAC AAG CGA CTC CAC TGT TAC GCG TCT TGG AGG AAC AAT	668
Gly Glu Lys Asp Lys Arg Leu His Cys Tyr Ala Ser Trp Arg Asn Asn	
55 60 65	
TCG GGC TTC ATA GAG CTG GTG AAA AAA GGA TGC TGG CTG GAT GAC TTC	716
Ser Gly Phe Ile Glu Leu Val Lys Lys Gly Cys Trp Leu Asp Asp Phe	
70 75 80	
AAC TGT TAT GAC AGA CAG GAA TGT ATT GCC AAG GAA GAA AAC CCC CAA	764
Asn Cys Tyr Asp Arg Gln Glu Cys Ile Ala Lys Glu Glu Asn Pro Gln	
85 90 95	

GTC TTT TTC TGC TGC TGC GAG GGA AAC TAC TGC AAC AAG AAA TTT ACT	812
Val Phe Phe Cys Cys Cys Glu Gly Asn Tyr Cys Asn Lys Lys Phe Thr	
100 105 110 115	
CAT TTG CCT GAA GTC GAA ACA TTT GAT CCG AAG CCC CAG CCG TCA GCC	860
His Leu Pro Glu Val Glu Thr Phe Asp Pro Lys Pro Gln Pro Ser Ala	
120 125 130	
TCC GTA CTG AAC ATT CTG ATC TAT TCC CTG CTT CCA ATT GTT GGT CTT	908
Ser Val Leu Asn Ile Leu Ile Tyr Ser Leu Leu Pro Ile Val Gly Leu	
135 140 145	
TCC ATG GCA ATT CTC CTG GCG TTC TGG ATG TAC CGT CAT CGA AAG CCT	956
Ser Met Ala Ile Leu Leu Ala Phe Trp Met Tyr Arg His Arg Lys Pro	
150 155 160	
CCC TAC GGG CAT GTA GAG ATC AAT GAG GAC CCC GGT CTG CCC CCT CCA	1004
Pro Tyr Gly His Val Glu Ile Asn Glu Asp Pro Gly Leu Pro Pro Pro	
165 170 175	
TCT CCT CTG GTC GGG CTG AAG CCG CTG CAG TTG CTG GAG ATA AAG GCG	1052
Ser Pro Leu Val Gly Leu Lys Pro Leu Gln Leu Leu Glu Ile Lys Ala	
180 185 190 195	
CGA GGC CGT TTC GGT TGC GTC TGG AAA GCT CGT CTG CTG AAT GAA TAT	1100
Arg Gly Arg Phe Gly Cys Val Trp Lys Ala Arg Leu Leu Asn Glu Tyr	
200 205 210	
GTC GCA GTG AAA ATC TTC CCC GTG CAG GAT AAG CAG TCG TGG CAG TGT	1148
Val Ala Val Lys Ile Phe Pro Val Gln Asp Lys Gln Ser Trp Gln Cys	
215 220 225	
GAG AAA GAG ATC TTC ACC ACG CCG GGC ATG AAA CAT GAA AAC CTA TTG	1196
Glu Lys Glu Ile Phe Thr Thr Pro Gly Met Lys His Glu Asn Leu Leu	
230 235 240	
GAG TTC ATT GCC GCT GAG AAG AGG GGA AGC AAC CTG GAG ATG GAG CTG	1244
Glu Phe Ile Ala Ala Glu Lys Arg Gly Ser Asn Leu Glu Met Glu Leu	
245 250 255	
TGG CTC ATC ACT GCA TTT CAT GAT AAG GGT TCT CTG ACG GAC TAC CTG	1292
Trp Leu Ile Thr Ala Phe His Asp Lys Gly Ser Leu Thr Asp Tyr Leu	
260 265 270 275	
AAA GGG AAC TTG GTG AGC TGG AAT GAA CTG TGT CAC ATA ACA GAA ACA	1340
Lys Gly Asn Leu Val Ser Trp Asn Glu Leu Cys His Ile Thr Glu Thr	
280 285 290	
ATG GCT CGT GGG CTG GCC TAC TTA CAT GAA GAT GTG CCC CGC TGT AAA	1388
Met Ala Arg Gly Leu Ala Tyr Leu His Glu Asp Val Pro Arg Cys Lys	
295 300 305	
GGT GAA GGG CAC AAA CCT GCA ATC GCT CAC AGA GAT TTT AAA AGT AAG	1436
Gly Glu Gly His Lys Pro Ala Ile Ala His Arg Asp Phe Lys Ser Lys	
310 315 320	
AAT GTA TTG CTA AGA AAC GAC CTG ACT GCG ATA TTA GCA GAC TTC GGG	1484
Asn Val Leu Leu Arg Asn Asp Leu Thr Ala Ile Leu Ala Asp Phe Gly	
325 330 335	
CTG GCC GTA CGA TTT GAG CCT GGA AAA CCT CCG GGA GAT ACA CAC GGG	1532
Leu Ala Val Arg Phe Glu Pro Gly Lys Pro Pro Gly Asp Thr His Gly	
340 345 350 355	
CAG GTT GGC ACC AGG AGG TAT ATG GCT CCT GAG GTT CTA GAG GGA GCA	1580
Gln Val Gly Thr Arg Arg Tyr Met Ala Pro Glu Val Leu Glu Gly Ala	
360 365 370	

ATT AAC TTT CAG CGA GAT TCC TTT CTC AGG ATA GAT ATG TAT GCC ATG Ile Asn Phe Gln Arg Asp Ser Phe Leu Arg Ile Asp Met Tyr Ala Met 375 380 385	1628
GGA CTG GTA CTC TGG GAA ATA GTA TCC CGA TGT ACA GCA GCA GAT GGG Gly Leu Val Leu Trp Glu Ile Val Ser Arg Cys Thr Ala Ala Asp Gly 390 395 400	1676
CCA GTA GAT GAG TAT CTG CTC CCA TTC GAA GAA GAG ATT GGG CAA CAT Pro Val Asp Glu Tyr Leu Leu Pro Phe Glu Glu Glu Ile Gly Gln His 405 410 415	1724
CCT TCC CTA GAG GAT CTG CAA GAA GTT GTC GTT CAC AAG AAG ATA CGC Pro Ser Leu Glu Asp Leu Gln Glu Val Val Val His Lys Lys Ile Arg 420 425 430 435	1772
CCT GTA TTC AAA GAC CAC TGG CTG AAA CAC CCT GGT CTG GCC CAA CTG Pro Val Phe Lys Asp His Trp Leu Lys His Pro Gly Leu Ala Gln Leu 440 445 450	1820
TGC GTC ACC ATT GAA GAA TGC TGG GAC CAT GAT GCG GAA GCA CGG CTT Cys Val Thr Ile Glu Glu Cys Trp Asp His Asp Ala Glu Ala Arg Leu 455 460 465	1868
TCG GCA GGC TGC GTA GAG GAG CGT ATT TCC CAA ATC CGT AAA TCA GTG Ser Ala Gly Cys Val Glu Glu Arg Ile Ser Gln Ile Arg Lys Ser Val 470 475 480	1916
AAC GGC ACT ACC TCG GAC TGC CTT GTA TCC ATT GTT ACA TCT GTC ACC Asn Gly Thr Thr Ser Asp Cys Leu Val Ser Ile Val Thr Ser Val Thr 485 490 495	1964
AAT GTG GAC TTG CCG CCC AAA GAG TCC AGT ATC TGAGGTTTCT TTGGTCTTTC Asn Val Asp Leu Pro Pro Lys Glu Ser Ser Ile 500 505 510	2017
CAGACTCAGT GACTTTTAAA AAAAAAATC ACGAATGCAG CTGCTATTTT ATCTTGACTT	2077
TTTAATATTT TTTTCTTGG ATTTACTTG GATCGGATCA ATTTACCAGC ACGTCATTCG	2137
AAAGTATTAA AAAAAA AAAACAAAA AAGCAAAAA AGACATCTCA GCAAGCATT	2197
AGGTGCCGAC TTATGAATGC CAATAGGTGC AGGAACTTCA GAACCTCAAC AAACCTATT	2257
CTAGAGAATG TTCTCTGGT TTCCTTTATC TCAGAAGAGG ACCCATAGGA AAACACCTAA	2317
GTCAAGCAAA TGCTGCAG	2335

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 510 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Gly Ala Ser Val Ala Leu Thr Phe Leu Leu Leu Leu Ala Thr Phe
1 5 10 15
Arg Ala Gly Ser Gly His Asp Glu Val Glu Thr Arg Glu Cys Ile Tyr
20 25 30

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Tyr Ala Met Gly Leu Val Leu Trp Glu Ile Val Ser Arg Cys Thr Ala
 385 390 395 400
 Ala Asp Gly Pro Val Asp Glu Tyr Leu Leu Pro Phe Glu Glu Glu Ile
 405 410 415
 Gly Gln His Pro Ser Leu Glu Asp Leu Gln Glu Val Val Val His Lys
 420 425 430
 Lys Ile Arg Pro Val Phe Lys Asp His Trp Leu Lys His Pro Gly Leu
 435 440 445
 Ala Gln Leu Cys Val Thr Ile Glu Glu Cys Trp Asp His Asp Ala Glu
 450 455 460
 Ala Arg Leu Ser Ala Gly Cys Val Glu Glu Arg Ile Ser Gln Ile Arg
 465 470 475 480
 Lys Ser Val Asn Gly Thr Thr Ser Asp Cys Leu Val Ser Ile Val Thr
 485 490 495
 Ser Val Thr Asn Val Asp Leu Pro Pro Lys Glu Ser Ser Ile
 500 505 510

SEQ ID NO.: 5

DLKPEN

SEQ ID NO.: 6

G(T/S)XX(Y/F)X

SEQ ID NO.: 7

DIKSKN

SEQ ID NO.: 8

GTRRYM

SEQ ID NO.: 9

DLAARN

SEQ ID NO.: 10

XP(I/V)(K/R)W(T/M)

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That which is claimed is:

1. A novel receptor protein characterized by having the following domains, reading from the N-terminal end of said protein:

5 an extracellular, ligand-binding domain,
a hydrophobic, trans-membrane domain, and
an intracellular, receptor domain having serine
kinase-like activity.

2. A protein according to Claim 1, further
10 comprising a second hydrophobic domain at the amino
terminus thereof.

3. A protein according to Claim 1, wherein said
protein is further characterized by having sufficient
15 binding affinity for at least one member of the
activin/TGF- β superfamily of polypeptide growth factors
such that concentrations of ≤ 10 nM of said polypeptide
growth factor occupy $\geq 50\%$ of the binding sites of said
receptor protein.

20

4. A protein according to Claim 3, wherein said
protein is further characterized by:

having a greater binding affinity for activins than
for inhibins,
25 having substantially no binding affinity for
transforming growth factors- β , and
having substantially no binding affinity for
non-activin-like proteins.

30

5. A protein according to Claim 1 having an
amino acid sequence substantially the same as set forth in
Sequence ID No. 2, Sequence ID No. 2', or Sequence ID No.

4.

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6. A soluble, extracellular, ligand-binding protein, further characterized by:

having a sufficient binding affinity for at least one member of the activin/TGF- β superfamily of polypeptide growth factors such that concentrations of ≤ 10 nM of said polypeptide growth factor occupy $\geq 50\%$ of the binding sites on said receptor protein, and

having at least about 30% sequence identity with respect to:

10 the sequence of amino acids 20-134 set forth in Sequence ID No. 2;

the sequence of amino acids 20-134 set forth in Sequence ID No. 2, wherein the arginine residue at position number 39 is replaced by a lysine, and the isoleucine at residue number 92 is replaced by a valine; or

15 the sequence of amino acids 21-132 set forth in Sequence ID No. 4.

20 7. A protein according to Claim 6, further characterized by:

having a greater binding affinity for activins than for inhibins,

25 having substantially no binding affinity for transforming growth factors- β , and

having substantially no binding affinity for non-activin-like proteins.

30 8. A protein according to Claim 6 wherein said protein comprises in the range of about 114-118 amino acids.

35 9. A DNA encoding a mature protein according to Claim 1.

10. A DNA encoding a mature protein according to Claim 3.

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11. A DNA encoding a precursor-form of the protein of Claim 1.

12. A DNA encoding a protein according to
5 Claim 2.

13. A DNA encoding a soluble protein according to Claim 6.

10 14. A DNA encoding a soluble protein according to Claim 8.

15 15. A DNA encoding a precursor-form of the protein of Claim 6.

16. A DNA according to Claim 9 having a contiguous nucleotide sequence substantially the same as:
nucleotides 128 - 1609 of Sequence ID No. 1;
variations of nucleotides 128 - 1609 of Sequence
20 ID No. 1, wherein the codon for residue number 39 of the encoded amino acid codes for lysine, the codon for residue number 92 of the encoded amino acid codes for valine, and the codon for residue number 288 of the encoded amino acid encodes glutamine;
25 nucleotides 528 - 1997 of Sequence ID No. 3; or variations of any of the above sequences which encode the same amino acid sequences, but employ different codons for some of the amino acids.

30 17. A DNA according to Claim 9 having a contiguous nucleotide sequence substantially the same as:
nucleotides 71 - 1609 of Sequence ID No. 1;
variations of nucleotides 71 - 1609 of Sequence
35 ID No. 1, wherein the codon for residue number 39 of the encoded amino acid codes for lysine, the codon for residue number 92 of the encoded amino acid codes for valine, and the codon for residue number 288 of the

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encoded amino acid encodes glutamine;
nucleotides 468 - 1997 of Sequence ID No. 3; or
variations of any of the above sequences which
encode the same amino acid sequences, but employ
5 different codons for some of the amino acids.

18. A DNA according to Claim 9 having a
contiguous nucleotide sequence substantially the same as
set forth in Sequence ID No. 1, Sequence ID No. 1' or
10 Sequence ID No. 3.

19. A DNA according to Claim 13 having a
contiguous nucleotide sequence substantially the same as
nucleotides 71 - 127 of Sequence ID No. 1, or nucleotides
15 468-527 of Sequence ID No. 3.

20. A method for the recombinant production of
activin receptor(s), said method comprising
expressing the DNA of Claim 9 in a suitable host cell.
20

21. A method for the recombinant production of
soluble activin receptor(s), said method comprising
expressing the DNA of Claim 13 in a suitable host
cell.
25

22. A DNA fragment useful as a hybridization
probe, wherein said DNA fragment comprises at least a
portion of the DNA according to Claim 9, and wherein said
DNA fragment is labeled with a readily detectable
30 substituent.

23. A DNA fragment according to Claim 22 wherein
said readily detectable substituent is selected from a
radiolabeled molecule, a fluorescent molecule, an enzyme,
35 or a ligand.

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24. A method to identify clones encoding receptors of the activin/TGF- β superfamily, said method comprising:

5 screening a genomic or cDNA library with a DNA fragment according to Claim 22 under low stringency hybridization conditions, and
identifying those clones which display a substantial degree of hybridization to said DNA fragment.

10

25. A method for screening a collection of compounds to determine those compounds which bind to receptors of the activin/TGF- β superfamily, said method comprising employing the receptor of claim 1 in a
15 competitive binding assay.

26. A bioassay for evaluating whether compounds are agonists for receptor protein(s) according to Claim 1,
20 or functional modified forms of said receptor protein(s), said bioassay comprising:

(a) culturing cells containing:

25 DNA which expresses said receptor protein(s) or functional modified forms of said receptor protein(s), and

DNA encoding a hormone response element operatively linked to a reporter gene,
wherein said culturing is carried out in the presence of at least one compound whose ability
30 to induce transcription activation activity of said receptor protein is sought to be determined;
and thereafter

(b) monitoring said cells for expression of said reporter gene.

35

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27. A bioassay for evaluating whether compounds are antagonists for receptor protein(s) according to Claim 1, or functional modified forms of said receptor protein(s), said bioassay comprising:

5 (a) culturing cells containing:

DNA which expresses said receptor protein(s), or functional modified forms of said receptor protein(s), and

10 DNA encoding a hormone response element operatively linked to a reporter gene;

wherein said culturing is carried out in the presence of:

15 increasing concentrations of at least one compound whose ability to inhibit transcription activation of said receptor protein(s) is sought to be determined, and

20 a fixed concentration of at least one agonist for said receptor protein(s), or functional modified forms of said receptor protein(s); and thereafter

(b) monitoring in said cells the level of expression of the product of said reporter gene as a function of the concentration of said compound, thereby indicating the ability of said compound to inhibit activation of transcription.

28. A method for modulating the transcription trans-activation of activin receptor(s), said method comprising:

30 contacting said receptor with an effective, modulating amount of the protein of Claim 6.

29. An antibody generated against the protein of Claim 6.

35

30. An antibody according to Claim 29, wherein said antibody is a monoclonal antibody.

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31. A method for modulating the transcription trans-activation of activin receptor(s), said method comprising:

contacting said receptor with a modulating, effective
5 amount of the antibody of Claim 29.

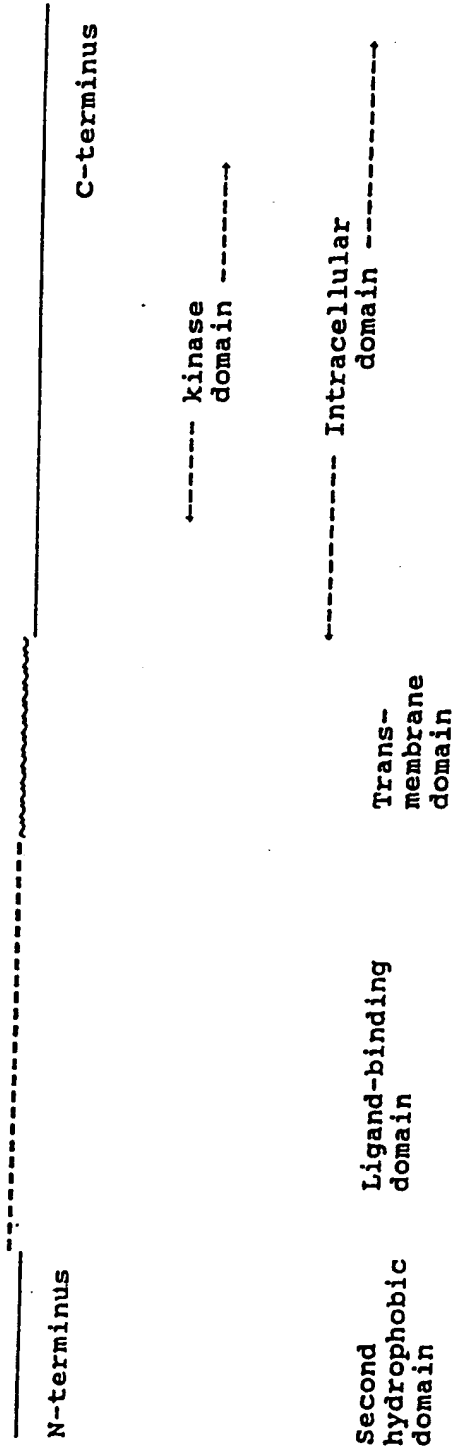


Figure 1

Divide a cDNA library in a mammalian expression vector into pools of 1000 clones, prepare DNA from each pool

Transfect COS cells directly on microscope slides

Bind [¹²⁵I] activin A, wash cells, fix, dip in photographic emulsion

Subdivide bacteria from positive pool and rescreen; repeat until receptor clone is pure

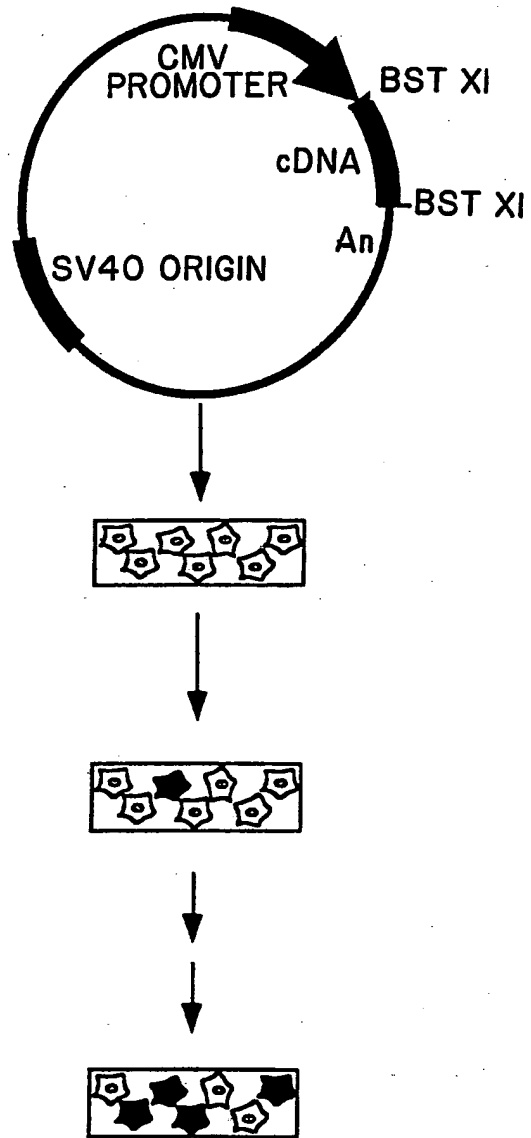


FIG. 2

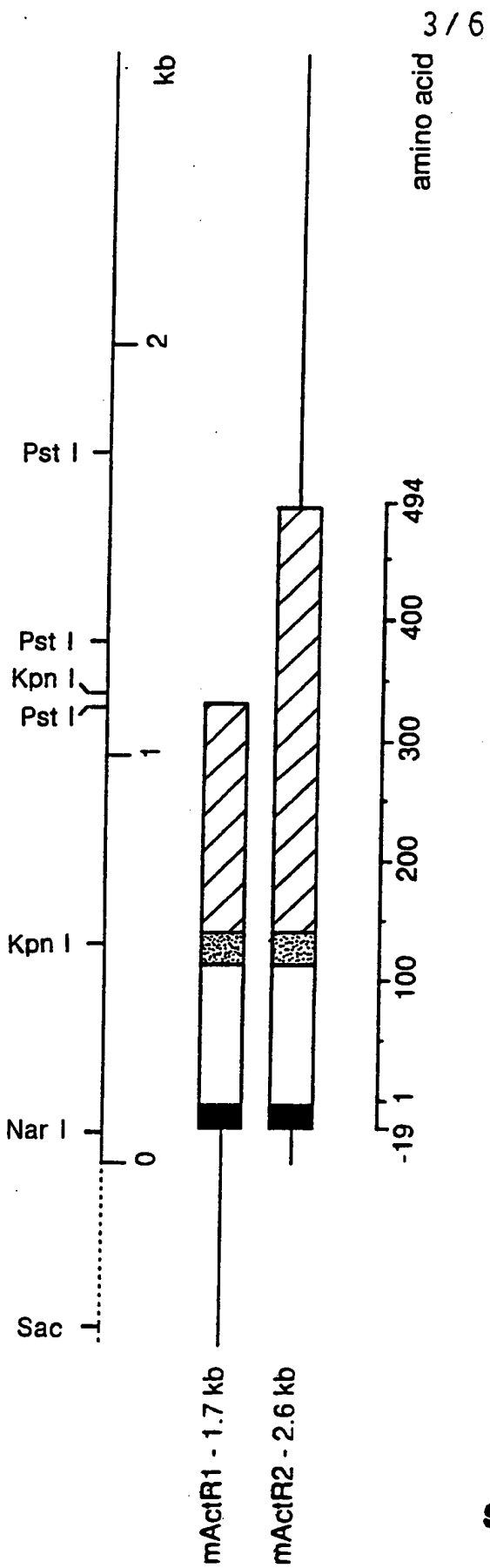


FIG. 3

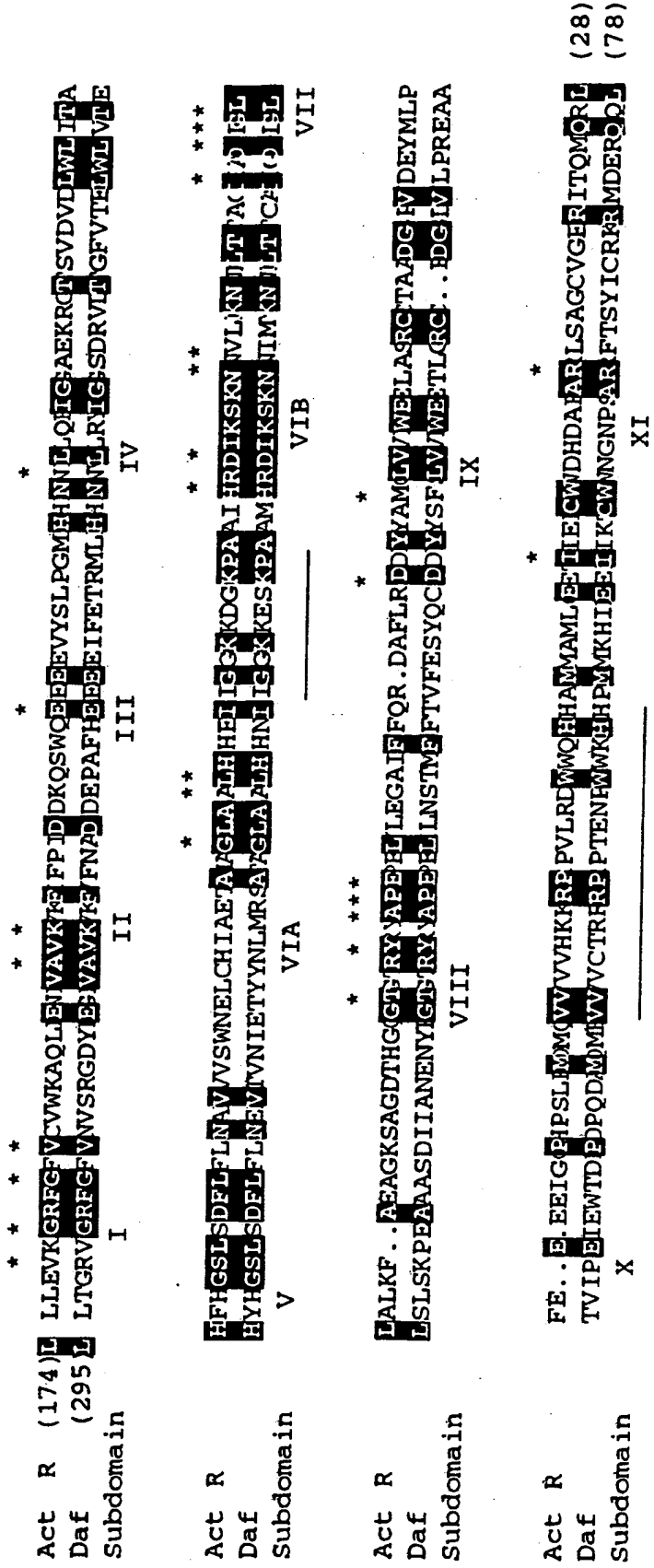
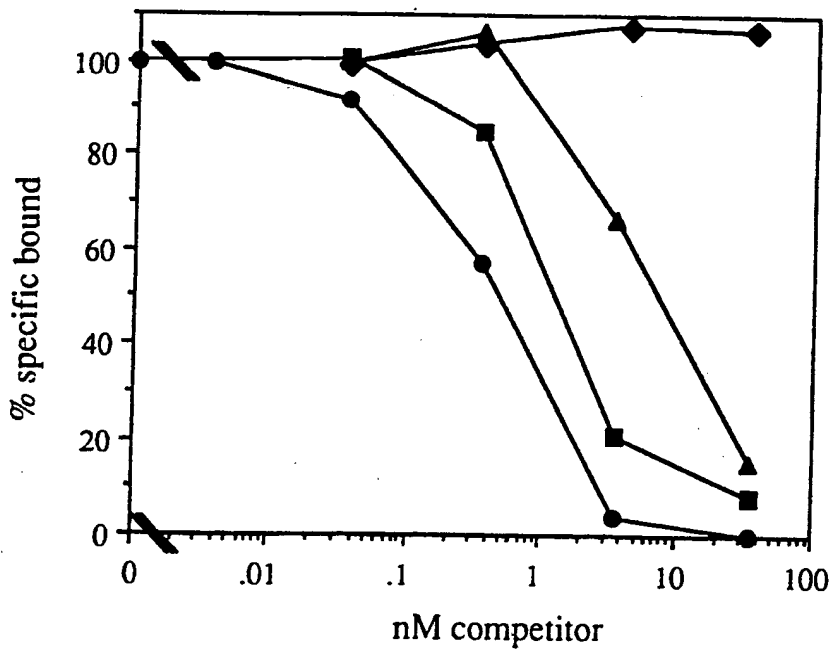
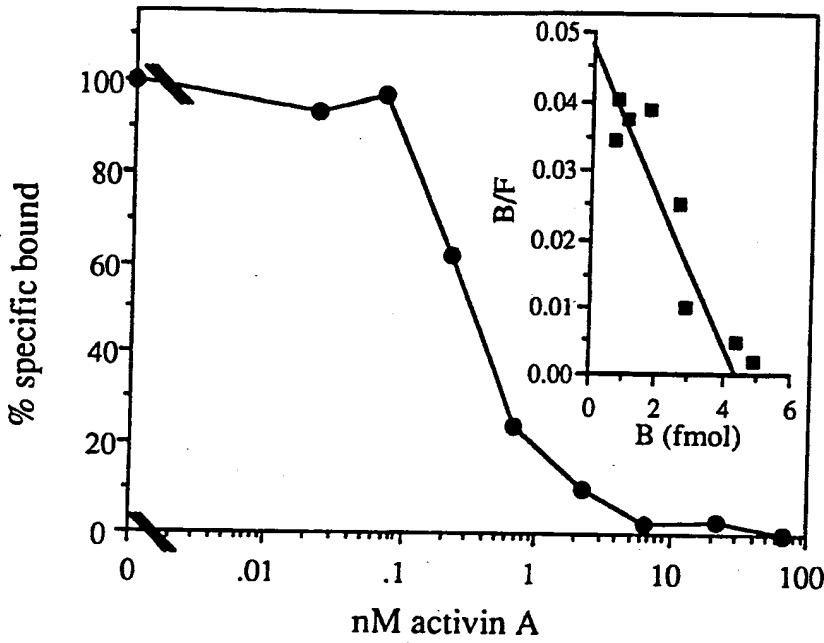


FIG. 4



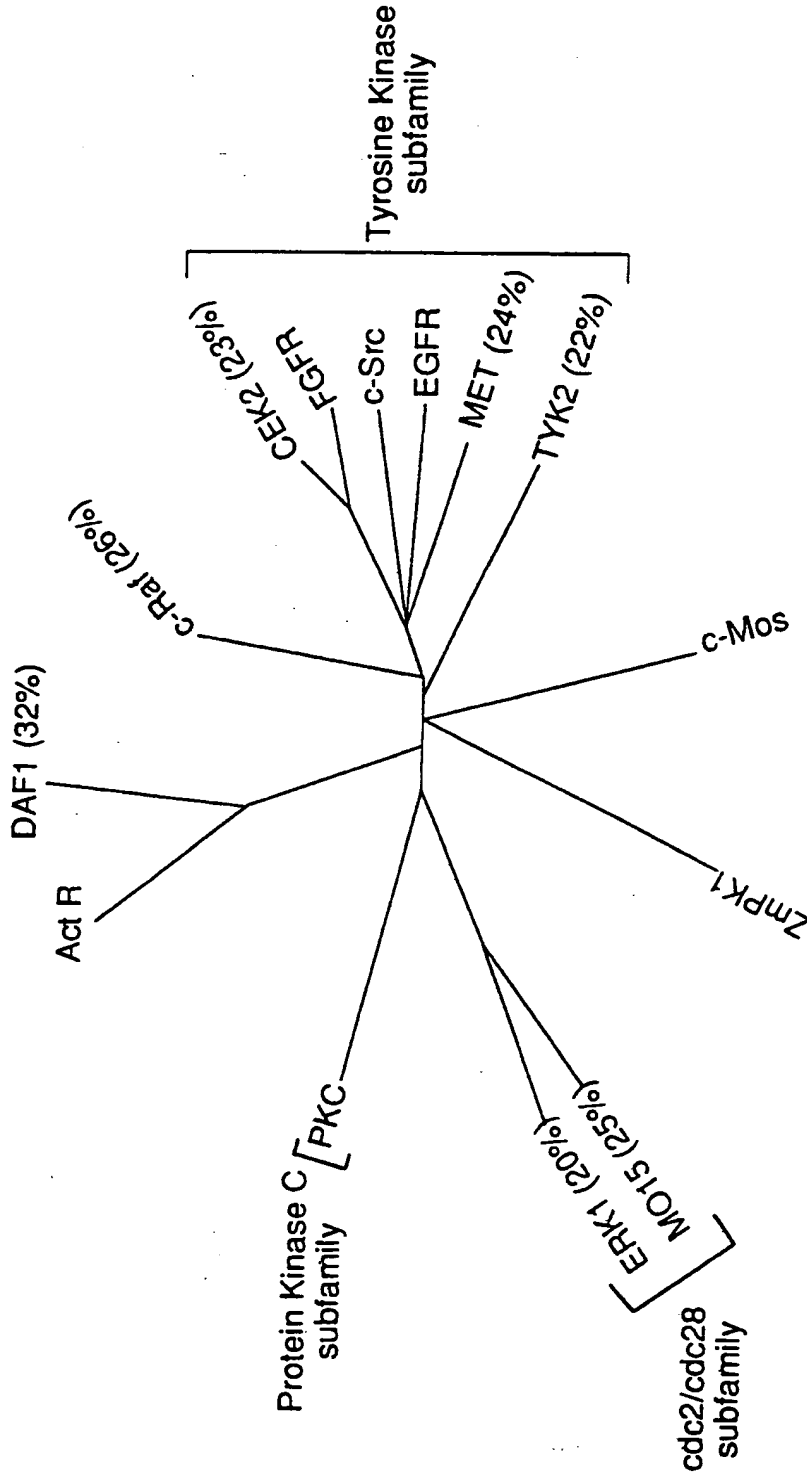
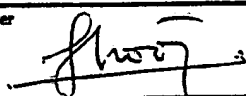


FIG. 6

INTERNATIONAL SEARCH REPORT

PCT/US 92/03825

International Application No

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶			
According to International Patent Classification (IPC) or to both National Classification and IPC			
Int.Cl. 5	C12N15/12; C12N15/62;	C07K15/00; A61K37/02;	C12Q1/68; C12P21/08;
			G01N33/53 A61K39/395
II. FIELDS SEARCHED			
Minimum Documentation Searched ⁷			
Classification System	Classification Symbols		
Int.Cl. 5	C12N ; G01N ;	C07K ; A61K	C12P ; C12Q
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸			
III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹			
Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²		Relevant to Claim No. ¹³
X	CELL vol. 61, no. 3, 18 May 1990, CAMBRIDGE, MA, US pages 635 - 645; L. GEORGI ET AL.: 'daf-1, a C. elegans gene controlling Dauer larva development, encodes a novel receptor protein kinase.' cited in the application see abstract; figures 4,6 ---		1,2,9, 11,12
X	BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS. vol. 157, no. 2, 15 December 1988, DULUTH, MN, US pages 844 - 849; C. CAMPEN ET AL.: 'Characterization of activin A binding sites on the human leukemia cell line K562.' see the whole document ---		1-4, 25
	-/-		
¹⁰ Special categories of cited documents : ^{"A"} document defining the general state of the art which is not considered to be of particular relevance ^{"E"} earlier document but published on or after the international filing date ^{"L"} document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) ^{"O"} document referring to an oral disclosure, use, exhibition or other means ^{"P"} document published prior to the international filing date but later than the priority date claimed		^{"T"} later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention ^{"X"} document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step ^{"Y"} document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. ^{"&"} document member of the same patent family	
IV. CERTIFICATION			
Date of the Actual Completion of the International Search		Date of Mailing of this International Search Report	
16 SEPTEMBER 1992		29. 09. 92	
International Searching Authority EUROPEAN PATENT OFFICE		Signature of Authorized Officer NOOIJ F.J.M. 	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		Relevant to Claim No.
Category ^a	Citation of Document, with indication, where appropriate, of the relevant passages	
P,X	<p>SCIENCE. vol. 255, no. 5052, 27 March 1992, WASHINGTON DC, US pages 1702 - 1705; L. MATHEWS ET AL.: 'Cloning of a second type of activin receptor and functional characterization in Xenopus embryos.' see abstract; figure 1</p>	1-5, 9-12,20, 25
P,X	<p>BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS. vol. 184, no. 1, 15 April 1992, DULUTH, MN, US pages 310 - 316; C. DONALDSON ET AL.: 'Molecular cloning and binding properties of the human type II activin receptor.' see abstract; figure 2</p>	1-5, 9-12, 16-20, 22-24
P,X	<p>CELL vol. 68, no. 4, 21 February 1992, CAMBRIDGE, MA, US pages 775 - 785; H. LIN ET AL.: 'Expression cloning of the TGF-beta type II receptor, a functional transmembrane serine/threonine kinase.' see abstract; figures 2,5,6</p>	1-3, 9-12, 22-24
P,X	<p>BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS. vol. 181, no. 2, 16 December 1991, DULUTH, MN, US pages 684 - 690; M. KONDO ET AL.: 'Activin receptor mRNA is expressed early in Xenopus embryogenesis and the level of the expression affects the body axis formation.' see abstract; figure 1</p>	1-5, 9-12, 16-18, 20,22-24

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 28 and 31 (both practically, as far as an in vivo method is concerned) are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
- No protest accompanied the payment of additional search fees.

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